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Molecular and isotopic characterisation of animal fats in archaeological pottery.

Molecular and Isotopic Characterisation of Animal Fats in Archaeological Pottery

by

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This thesis is submitted to the Faculty of Science of the University of Bristol, U.K.

In fulfilment of the requirements for the degree of Doctor of Philosophy

DECLARATION

I certify that the work described herein is my own, except where otherwise stated, and has not been submitted for a degree at this, or any other university

Stephanie Noelle Dudd

To Gareth Rieley

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ABSTRACT

The molecular and isotopic compositions of modern fats and lipid residues associated with archaeological pot sherds have been compared in order to draw distinctions between diagenetically altered fats derived from the major domesticated animal species exploited in prehistory. The study has employed a range of analytical techniques: GC and GC/MS for the determination of overall lipid distributions, relative abundances of free fatty acids including positional and geometric isomers of the mono-unsaturated C_{18} components, and triacylglycerol distributions; GC-C-IRMS for the measurement of stable carbon isotope ratios of individual *n*-alkanoic acids.

The investigation has enabled clear distinctions to be drawn between diagenetically altered fats derived from ruminant and non-ruminant animals. The major alterations observed in decayed fats have occurred as a result of the hydrolysis of acyl lipids and β- and autoxidation of unsaturated lipid components during vessel use and burial. Following hydrolysis, the lower carbon-number fatty acids such as the $C_{4:0}$ to $C_{14:0}$ components diagnostic of fresh dairy fats are preferentially lost. The transformation of the lipid distribution of milk fat during decay to one resembling ruminant adipose fat has been demonstrated through laboratory decay experiments, explaining why degraded dairy fats have thus far been difficult to detect in the archaeological record. In addition, the higher stability of the *trans*-isomers of the $C_{18:1}$ fatty acid is evident due to the preferential loss of *cis*-configured components from archaeological fats. Laboratory degradation experiments have demonstrated that the incorporation of bacterial fatty acids during decay is minimal.

Comparison of robust $\delta^{13}C$ values of saturated $C_{16:0}$ and $C_{18:0}$ fatty acids in modern and degraded fats, e.g. from laboratory decay experiments, has enabled distinctions to be drawn between degraded ruminant and non-ruminant fats. Differences in their stable isotope compositions are based upon metabolic and physiological variations and dietary preferences. Furthermore, distinctions have been drawn between ruminant dairy and adipose fats, due to biases in the biosynthetic origins of the fatty acids in milk and adipose fat which result in a more depleted isotopic signal (*ca.* 2-4‰) for the $C_{18:0}$ fatty acid in ruminant milk fat. The distinction between ruminant and non-ruminant fats is supported by characteristic carbon-number ranges and relative abundances of intact triacylglycerols where they are preserved in archaeological residues. Porcine adipose fats are relatively enriched in ^{13}C ($C_{18:0}$ = *ca.* -25‰) compared to ruminant fats ($C_{18:0}$ = *ca.* -32‰) and are characterised by a narrow distribution of triacylglycerols, ranging between C_{44} and C_{54}. In contrast, ruminant adipose fat triacylglycerols range between C_{42} and C_{54}. Intact triacylglycerols ranging from C_{40} to C_{54} have been detected in well-preserved dairy fats. Distinctions are also supported by the relative abundances of the Δ^{10} and Δ^{11} *cis*- and *trans*-configured $C_{18:1}$ isomers, with a high abundance of *trans*-Δ^{11} indicating a ruminant fat. Caution is required in using branched-chain fatty acids as a diagnostic criterion for ruminant fats due to their direct transfer from the diet into the body fats of non-ruminant animals.

The analysis of residues from a wide range of unglazed vessel forms dating from the Saxon and medieval through to the Iron Age and middle Neolithic periods has shown the high frequency with which lipid components are preserved by entrapment within the ceramic matrix of pottery vessels. The origins assigned to the archaeological fats from the late Saxon/early medieval site of West Cotton, Northamptonshire on the basis of the chemical analyses correlate with the documentary and faunal evidence which indicate the importance of sheep at the site. The identification of non-ruminant fats in Saxon pottery from Wicken Bonhunt, Essex, correlates with the preponderance of pig bone excavated from the site. The results obtained validate the methods used in assigning origins to archaeological fats. The techniques have also been successfully applied to earlier assemblages, including Upper Ninepence, a Neolithic settlement in the Welsh borderlands, where no faunal or environmental remains have survived. The molecular and isotopic information obtained from the pottery residues provides the only evidence for the exploitation of animals for their meat and milk at this site. The identification of dairying as an element of prehistoric economies has opened the way for the study of the exploitation of dairy products amongst ancient populations.

ABBREVIATIONS

DAF	Degraded animal fat
DAG	Diacylglycerol
DMDS	Dimethyl disulphide
FAME	Fatty acid methyl ester
FFA	Free fatty acid
GC	Gas chromatography
GC-C-IRMS	Gas chromatography-combustion-isotope ratio mass spectrometry
GC/MS	Gas chromatography/mass spectrometry
HTGC	High temperature gas chromatography
MAG	Monoacylglycerol
E/M/LIA	Middle/early/late Iron Age
E/M/LBA	Middle/early/late Bronze Age
TAG	Triacylglycerol
TLC	Thin layer chromatography
TLE	Total lipid extract
TMS	Trimethylsilyl
UCM	Unresolved complex mixture

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CHAPTER 1
Introduction

1.1 Farming in Prehistory

The introduction of agriculture into Europe in the 5th millennium BC involved both the migration of farmers and the diffusion of livestock and ceramics into an indigenous hunter-gatherer society (Keeley, 1992). It is believed that the major factor which had to change to make domestication possible was that of human attitudes and human behaviour, requiring a state of social co-operation and the domestication of humans themselves before the domestication of animals (Reed, 1987). The bulk of information presently known about animal exploitation in prehistory has been derived from the analysis of faunal assemblages from archaeological sites, and since remains of aurochs (*Bos primigenius*), forest bisons (*Bison bonasus*), brown bears (*Ursus arctos*) and wolves (*Canis lupus*) are uncommon in early Neolithic and Mesolithic contexts (Boessneck *et al.*, 1963; Bogucki, 1982), it is assumed they were not hunted on a regular basis, however, commonly occurring wild resources included red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*), boar (*Sus scrofa scrofa*) and beaver (*Castor fiber*), as well as fish, birds and small game (Hayes, 1993).

Neolithic Europeans are believed to have farmed cattle, goats, pigs and sheep, although horses were not yet domesticated. The hooved mammals (ungulates) are classified as shown in Table 1.1. Early Neolithic sheep had hair, but they had not yet developed woolly fleeces and it is therefore assumed that ovicaprids were kept primarily for milk (Gregg, 1988). Cattle would have provided a source of meat, milk, blood, leather and bone, as well as acting as beasts of burden and although early Neolithic pigs were small, averaging approximately 30 kg, they would have reproduced quickly and converted village waste into an excellent source of meat and fat. It is likely, however, that pig husbandry became effective and profitable only after the emergence of cultivation, because they could be fed on agricultural by-products (Kim, 1994). It would be another millennium before either ducks or cats were domesticated and several millennia before chickens were introduced to Europe (Gregg, 1988).

Macrobotanical evidence indicates that Neolithic domestic crops were limited to wheat, peas, lentils and flax and occasionally poppy, with barley rarely occurring at early Neolithic sites. Hazel shrubs and oak provided nuts until the widespread dominance of the

beech trees in the middle Neolithic and the introduction of chestnut trees in the Roman period. A variety of raspberries, strawberries, elderberries and vibernums would have been available along the forest margins or in clearings and wild onions and wild garlic were present in the deciduous forest (Gregg, 1988).

Table 1.1 Characteristics of the major ungulates domesticated in prehistory.

Order	Perissodactyla (odd toed)	Artiodactyla (even toed)		
Sub order	Hippomorpha	Suina	Ruminantia (true ruminants)	
Family	Equidae (**horse**)	Suidae (**swine**)	Cervidae (**deer**)	Bovidae
Genera				Bos (**cattle**) Capra (**goat**) Ovix (**sheep**)
Microbial fermentation occurs in:	Greatly enlarged caecum and colon	Enlarged caecum and colon plus large non-secretory area in stomach	Three compartments in stomach (reticulum, rumen, omasum)	

Although the identity of many available plant and animal resources in Neolithic and later periods has already been established, actual patterns of exploitation of the various natural commodities are more difficult to interpret due to biases and taboos in the diet and distribution of resources. Currently, the use of different commodities, e.g. in the domestic situation, and the functional significance of different ceramic vessel forms in processing, is generally inferred from indirect evidence or based on assumption and comparisons with ethnographic accounts. For example, in Turan (Iran) goats are the main milk producers because they are in milk from late February to late September; some cows are kept for milking during winter and in the summer cow's milk is often combined with sheep and goat milk for processing (Martin, 1980). Milk is heated to convert it to yoghurt and clarified butter. Liquid stored in skins and porous pottery vessels is kept cool since moisture seeps out through the material and evaporates. Due to the different emphasis placed on the importance of particular species by people of different races and cultures, the question of resource utilisation in prehistory would benefit from the identification of animal products themselves in order to obtain direct information relating to animal exploitation, consumption patterns and manufacturing in the ancient past.

1.2 Chemical analysis of archaeological pottery residues

The day to day use of pottery in the processing, transport and storage of natural materials in antiquity, and the actions of heating, grinding and pounding, would have facilitated the absorption of fats, oils and waxes into the walls of unglazed vessels (Evershed, 1993; Heron and Evershed, 1993). The clay microstructure affords protection from the burial environment such that the original nature of the commodities processed can be identified many thousands of years later. Although absorbed residues are the most common find, their preservation facilitated by the durability of the pottery haven, information has also been obtained from the analysis of carbonised residues adhering to the inner or outer surfaces of sherds. The analyses carried out on carbonised surface residues by Rottländer and Schlichtherle (1979), Needham and Evans (1987), Gurfinkle and Franklin (1988), Rottländer (1990), and Hill and Evans (1989) constitute some of the earliest analyses of organic residues associated with pottery vessels.

The most common class of analytes studied in residue analysis are the solvent extractable lipids, since their non polar, hydrophobic nature means they are in general less prone to decay and dissolution than, for example, proteins or carbohydrates, over archaeological time. Lipid residues have been used to derive information relating to the nature of commodities processed or stored in vessels during their use (Evershed *et al.*, 1991, 1992a,b, 1994; Charters *et al.*, 1995; Heron and Evershed, 1993; Condamin *et al.*, 1976) and in determining the actual mode of use of individual vessels (Charters *et al.*, 1993b, Evershed *et al.*, 1995a). Extensive work carried out recently on a largely domestic assemblage from the site of West Cotton, Northamptonshire, has established the advantages of carrying out large numbers of analyses from one site, providing a unique insight into the modes of vessel use and dietary preferences of a late Saxon/early medieval society in middle England (Charters, 1996).

1.3 Analytical techniques

Various analytical techniques have been utilised in recent years in order to classify organic residues into groups of broadly similar chemical composition, e.g. gas chromatography (GC; Condamin *et al.*, 1976), high performance liquid chromatography (HPLC; Passi *et*

al., 1981), bulk isotopic analysis (DeNiro, 1987), infrared spectroscopy (IR; Badler, 1990), high temperature gas chromatography (HTGC) and HTGC/mass spectrometry (HTGC/MS; Evershed *et al.*, 1990), GC-combustion-isotope ratio MS (GC-C-IRMS; Evershed *et al.*, 1994) and solid state ^{13}C nuclear magnetic resonance spectroscopy (NMR; Sherriff *et al.*, 1995). The most common approach to identifying the commodity originally processed is by drawing comparisons between the type and relative abundance of individual components present in the solvent extracts of the archaeological vessels with those which characterise modern reference materials. HTGC has been very successfully applied to the analysis of archaeological samples, enabling detailed compositional information to be obtained on a broad range of lipid classes without the need to chemically degrade intact lipids to their lower molecular weight component parts thus losing valuable diagnostic information (Evershed *et al.*, 1990, 1994, 1997b).

1.4 The interpretation of degraded lipid profiles and the identification of commodities.

The identification of ancient commodities from lipid residues in pottery is inevitably complicated by the degradative processes occurring during vessel use and burial, however, reliable identifications can be made based on the occurrence of diagnostic components and comparison of lipid profiles with modern reference samples and degraded materials produced in decay experiments carried out in the laboratory (Evershed *et al.*, 1995a).

Degraded animal fats are characterised by a readily recognisable distribution of free fatty acids, mono-, di- and triacylglycerols which can be identified using HTGC and HTGC/MS (Evershed, 1993; Evershed *et al.*, 1992a,b, 1995a). The laboratory decay of lamb fat has shown the pattern of lipid components produced when the intact triacylglycerol components of animal fats are hydrolysed (Evershed *et al.*, 1995a). Figure 1.1 illustrates the lipid profile of a degraded animal fat extracted from the rim sherd of a Romano-British vessel (sample ST206) from Stanwick, Northamptonshire (Dudd and Evershed, unpublished data). The fatty acids in degraded animal fats may range from $C_{8:0}$ to $C_{20:0}$, with the $C_{16:0}$ and C_{18} components predominating. Mono- and diacylglycerols produced by the loss of two or one fatty acid(s), respectively, are often low in abundance since complete

hydrolysis is rapid following the loss of one fatty acid. Animal fat triacylglycerols commonly range between C_{40} and C_{54}, with the C_{50} and C_{52} components the most abundant. Cholesterol is the major sterol present in animal tissues and is frequently identified in low abundance in degraded animal fat residues.

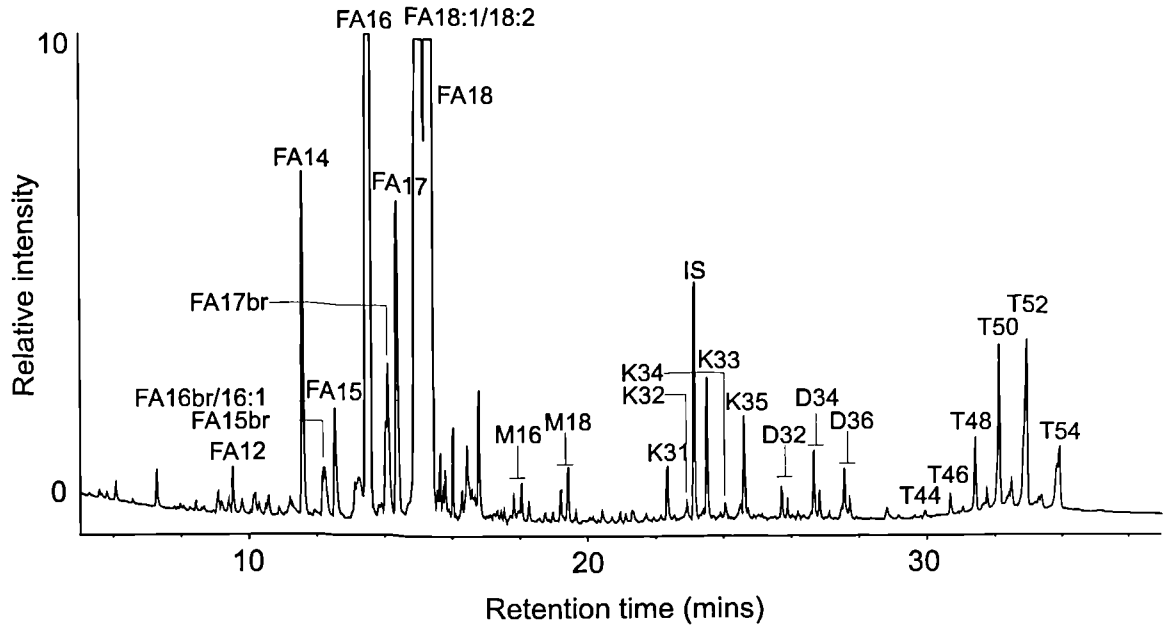


Figure 1.1 Partial HTGC profile of the trimethylsilylated extract from a Romano-British sherd (ST206) from Stanwick, Northamptonshire. The analysis was performed on a 15 m x 0.32 mm ID fused silica capillary column coated with HP1 stationary phase (immobilised dimethyl polysiloxane; 0.1 μm film thickness; J&W Scientific) using hydrogen as carrier gas (column head pressure 10 psi). The temperature programme consisted of a 2 minute isothermal at 50°C, then 50 to 350°C at 10°C min^{-1}, followed by a 10 min hold at 350°C. Sample introduction was by on-column injection. Note that the major peaks in the chromatogram have been expanded off scale to reveal detail of the minor constituents. Peak identities are: FA12, FA14, FA15, etc. correspond to *n*-alkanoic acids with 12, 14 and 15 carbon atoms, etc., respectively; FA17br refers to a branched-chain alkanoic acid with 17 carbon atoms; FA16:1 and FA18:1 refer to monounsaturated *n*-alkanoic acids containing 16 and 18 carbon atoms, respectively; M16 and M18 refer to monoacylglycerols containing 16 and 18 acyl carbon atoms, respectively (the 1-isomer elutes before the 2-isomer); K31, K32, etc. refer to mid-chain ketones formed by a condensation reaction involving two fatty acids (Evershed *et al.*, 1995; Raven *et al.*, 1997; discussed further in Chapter 3); D30, D32, etc. refer to diacylglycerols containing 30, 32, etc. acyl carbon atoms, respectively (the 1,2-isomer elutes before the 1,3-isomer); T44, T46, T48, etc. correspond to triacylglycerols bearing 44, 46, 48, etc. acyl carbon atoms, respectively; IS = internal standard (*n*-tetratriacontane) added at the extraction stage to enable quantification of lipid. All peak assignments have been confirmed by GC/MS analysis.

Commodities other than animal fats can also be identified on the basis of distributions of lipid components, with the most commonly recognised natural products including beeswax (Charters *et al.*, 1995; Needham and Evans, 1987; Evershed *et al.*, 1997b), birch bark tar (Charters *et al.*, 1993a; Dudd and Evershed, 1999), the epicuticular waxes of leafy vegetables (Charters *et al.*, 1997; Evershed *et al.*, 1991, 1992a,b, 1994) and olive oil (Condamin *et al.*, 1976).

Biomolecular information on leafy plant and vegetable matter is scarce in association with absorbed pottery residues, however the components comprising leaf waxes, i.e. the wax ester components, are relatively resistant to decay and components ranging between C_{42} and C_{50} have been identified in vessels from the Late Saxon/early medieval assemblage from West Cotton, Northamptonshire (Charters, 1996). Sixty percent of potsherds from Romano-British Mortaria from Stanwick, Northamptonshire, have been found to contain plant leaf wax constituents, including wax esters, alkanes, alcohols ranging from C_{24} to C_{32}, long-chain ketones and sterols, including sitosterol and stigmasterol (Dudd and Evershed, unpublished data). The C_{29} and C_{31} ketones have been identified in various Romano-British vessel types, including Mortaria and Cream Ware and Grey Ware dishes. GC/MS of the wax ester components identified in Mortaria from Stanwick (Sample 115; 140-180 AD; Dudd and Evershed, unpublished data), has shown the distribution of components is far from simple, with a mixture of three C_{46} components eluting at the same GC retention time, comprising C_{16}, C_{18} and C_{20} fatty acids and C_{30}, C_{28} and C_{26} alcohols, respectively. Leafy vegetable residues have been identified in Late Saxon/early medieval 'cooking' vessels from West Cotton due to the presence of three diagnostic components in a distribution characteristic of *Brassica* leaf waxes (Fig. 1.2). These components included *n*-nonacosane, nonacosan-15-one and nonacosan-15-ol and were first identified in association with pottery vessels by Evershed *et al.* (1991). Stable carbon isotope analysis has confirmed the C_3-plant origin of these components (Evershed *et al.*, 1994). Charters (1996) also noted the presence of the C_{31} ketone, together with hentriacontane (C_{31} *n*-alkane) in a Lyveden A ware jar from West Cotton. These components are characteristic of the epicuticular leaf waxes of modern leek (*Allium porrum*; Evershed *et al.*, 1995b; Raven, 1995). The identification of residues of *Brassica* and *Allium sp.* at West Cotton is consistent with documentary evidence which relates to dietary habits of the period (Henisch, 1976).

Interestingly, to date, no evidence of leafy vegetable or plant material has been identified in any pottery vessels dating before the Romano-British period in the UK which may suggest different methods of processing these types of commodities prior to this period (Dudd and Evershed, unpublished data). Mixtures of degraded animal fats and plant leaf wax components have been identified in 'cooking' vessels from both West Cotton and Stanwick, however it is unclear whether the commodities were being processed simultaneously or whether the vessels had multiple uses.

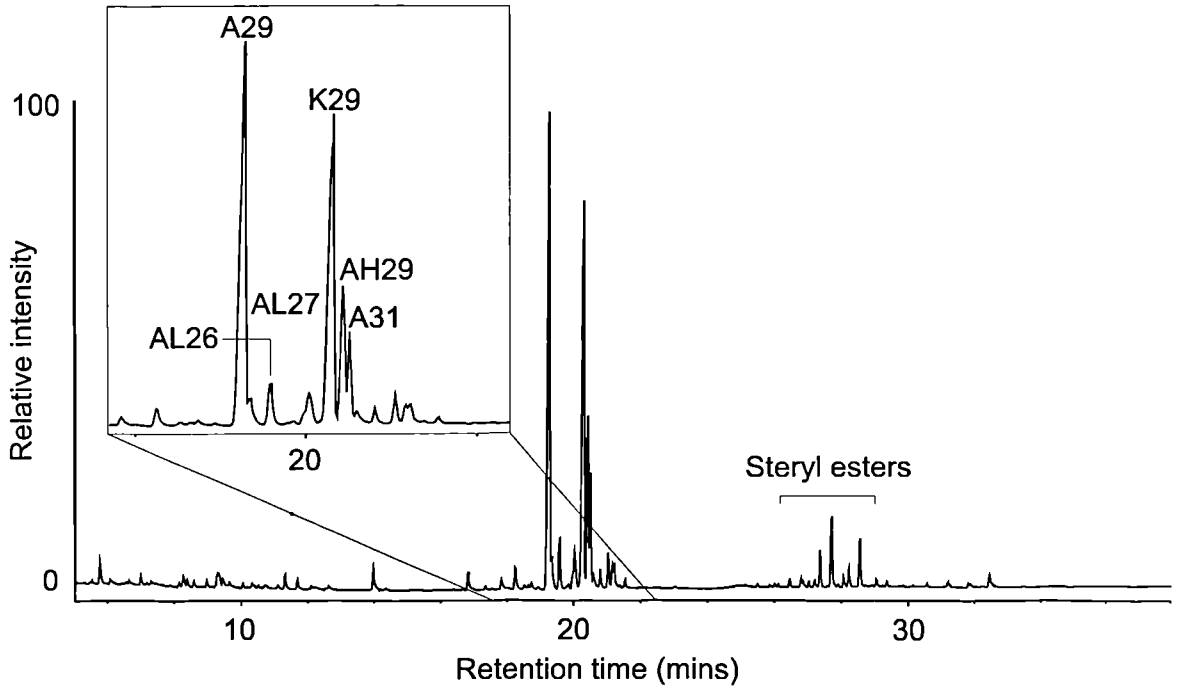


Figure 1.2 Trimethylsilylated total lipid extract of a Late Saxon/early medieval 'cooking' vessel from West Cotton. The analysis was performed under the same conditions as described in Figure 1.1. The distribution of components is characteristic of *Brassica* leaf wax. Peak identities are: A29 = *n*-nonacosane (C_{29}) and A31 = *n*-hentriacontane (C_{31}); AL26 = *n*-hexaeicosene (C_{26}) and AL27 = *n*-heptaeicosene (C_{27}); K29 = nonacosan-15-one (C_{29}); AH29 = nonacosan-15-ol (C_{29}).

Intact beeswax is characterised by a series of wax esters ranging from C_{38} to C_{52}, with the C_{46}, C_{48}, C_{40}, C_{42} and C_{44} esters predominating (Kolattukudy, 1976; Mills and White, 1994). The wax esters in beeswax can be distinguished from those derived from leafy vegetables since they are predominantly comprised of $C_{16:0}$ (although with lesser amounts of $C_{18:1}$, $C_{18:0}$ and $C_{20:0}$) esterified to alcohols ranging from C_{24} to C_{34}. Figure 1.3 shows a lipid extract of 100 year-old beeswax from Crete. The residue also contains a range of long-chain alkanes and fatty acids ranging between C_{23}-C_{33} and C_{22}-C_{34}, respectively. Decay results in

hydrolysis of the esters to release long-chain alcohols, as seen in the lipid extract of a conical cup from Late Minoan Crete (Evershed *et al.*, 1997b). Degraded beeswax residues have also been identified in coarse ware vessels believed to have been used as beehives at Isthmia in ancient Greece (Evershed *et al.*, in prepn.).

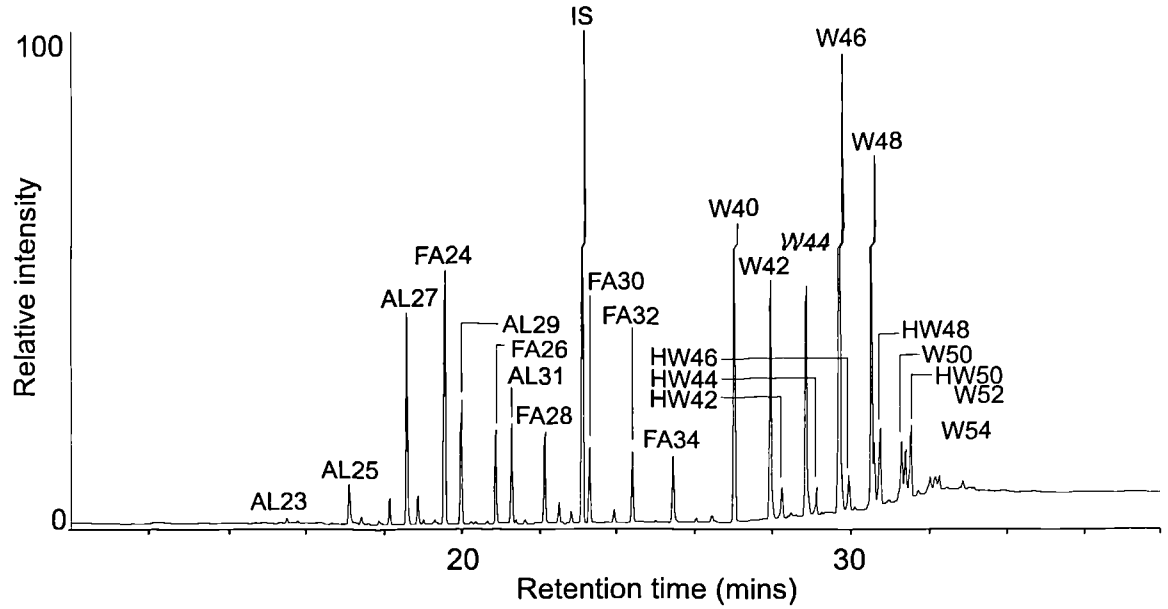


Figure 1.3 Trimethylsilylated total lipid extract of 100 year old beeswax from Crete (Evershed *et al.*, 1997b). The analysis was performed under the same conditions as described in Figure 1.1. Peak identities: FA24-FA34, saturated fatty acids bearing 24-34 carbon atoms, respectively, FA24 = tetracosanoic acid (C_{24}); FA26 = hexacosanoic acid (C_{26}); FA28 = octacosanoic acid (C_{28}); FA30 = triacontanoic acid (C_{30}); FA32 = dotriacontanoic acid (C_{32}); FA34 = tetratriacontanoic acid (C_{34}); AL23-AL31, *n*-alkanes containing 23-31 carbons, respectively, AL23 = triacosane (C_{23}); AL25 = pentacosane (C_{25}); AL27 = heptacosane (C_{27}); AL29 = nonacosane (C_{29}); AL31 = hentriacontane (C_{31}); IS, internal standard, *n*-tetratriacontane (C_{34}); W40-W54, wax esters containing 40-54 carbons, respectively, W40 = tetracosanyl palmitate (C_{40}); W42 = hexacosanyl palmitate (C_{42}); W44 = octacosanyl palmitate (C_{44}); W46 = triacontanyl palmitate (C_{46}); W48 = dotriacontanyl palmitate (C_{48}); W50 = tetratriacontanyl palmitate (C_{50}); W52 = hexatriacontanyl palmitate (C_{52}); W54 = octacontanyl palmitate; HW42-HW50, hydroxy-fatty acid-wax esters containing 42-50 carbons, respectively, HW42 = hexacosanyl hydroxy-palmitate (C_{42}); HW44 = octacosanyl hydroxy-palmitate (C_{44}); HW46 = triacontanyl hydroxy-palmitate (C_{46}); HW48 = dotriacontanyl hydroxy-palmitate (C_{48}); HW50 = tetratriacontanyl hydroxy-palmitate (C_{50}), and HW52 = hexatriacontanyl hydroxy-palmitate (C_{52});

Fresh olive oil is composed mainly of mixed triacylglycerols comprising C_{16} and C_{18} fatty acids. $C_{18:1}$ is the major component in the saponified oil, comprising 55-85%, followed by $C_{16:0}$, $C_{18:2}$, $C_{16:1}$ and $C_{18:0}$, the latter comprising only 0.5-4% (Condamin *et al.*, 1976). Olive

oil has been tentatively identified in a classical Roman lamp from Isthmia, Cyprus (Evershed, Dudd, Rush and Gaber, unpublished data) and in Roman amphorae (Condamin *et al.*, 1976).

An adhesive used to repair an Ecton ware jar recovered from Roman sediments of the River Nene at West Cotton, Northamptonshire, has been identified as birch bark tar (Charters *et al.*, 1993a). Birch bark and other tars are believed to have had a wide variety of uses in antiquity (Mills and White, 1994) due to their waterproofing and adhesive properties. Birch bark tar contains a high abundance of betulin, with lupenone and lupeol as minor constituents (Hayek *et al.*, 1990; O'Connell *et al.*, 1998). Examples of archaeological birch bark tars have also been described by Binder *et al.* (1990), Reunanen *et al.* (1993) and Dudd and Evershed (1999).

Although the identification of a residue as either a fat, oil or wax is relatively straightforward, the identification of the particular type of animal or plant from which the fat or oil is derived is more difficult, complicated to some extent by chemical and micro-biological alteration (Evershed *et al.*, 1992a). Such alterations are probably best assessed through the laboratory decay of lipids absorbed in potsherds; these are discussed further in Chapter 7. Since such a high proportion of the residues which have been analysed in our laboratory to date are derived from animal fats, extremely useful information would be obtained if we could distinguish between degraded animal fats of different origins. Our inability to do this has been compounded by the fact that the unsaturated components of animal fats which are often the most diagnostic in modern fats (Matter, 1992; Matter *et al.*, 1989) are much less likely to survive on archaeological time scales than their saturated counterparts due to their higher chemical reactivity (Frankel, 1980). Furthermore, animal fats invariably consist of similar distributions of the same range of components, e.g. free fatty acids, mono-, di- and triacylglycerols, so ruling out the possibility of using diagnostic biomarker components in making distinctions.

There are few reports of the identification of dairy fats associated with archaeological artefacts, however, in Rottländer's seminal publication in *Archaeo-Physika* (Rottländer, 1990), 6 milk or butter fats are tentatively identified from the total of >150 archaeological

animal fats reported. Likewise, Rottländer and Schlichtherle (1979) tentatively identified 4 cases of milk or butter fat in carbonised deposits from Neolithic pottery. The identification of milk fats relies on detecting the presence of short-chain, saturated fatty acids containing less than 14 carbon atoms ($<C_{14}$), in addition to the $C_{16:0}$ and $C_{18:0}$ components that dominate adipose fats. In fresh milk, the shorter-chain components ($C_{4:0}$ to $C_{12:0}$) typically account for up to 20% of the total fatty acid content (McDonald *et al.*, 1988). Significantly, the low incidence of milk fats reported by Rottländer is of the same order as our own findings. The HTGC profile of a pottery extract which appears to represent a degraded milk fat is shown in Figure 1.4. As discussed above, the identification of milk fat in this example is based on the presence of the lower carbon-number fatty acid components seen eluting at short retention times (3 to 10 mins). These $C_{8:0}$ to $C_{12:0}$ components are also detectable by GC/MS as acyl moieties amongst the intact mono-, di- and triacylglycerols (Evershed *et al.*, 1992b), and in intact cholesteryl fatty acyl esters purified from the TLE by thin layer chromatography (TLC) and characterised using negative ion ammonia chemical ionisation GC/MS (Evershed, 1994).

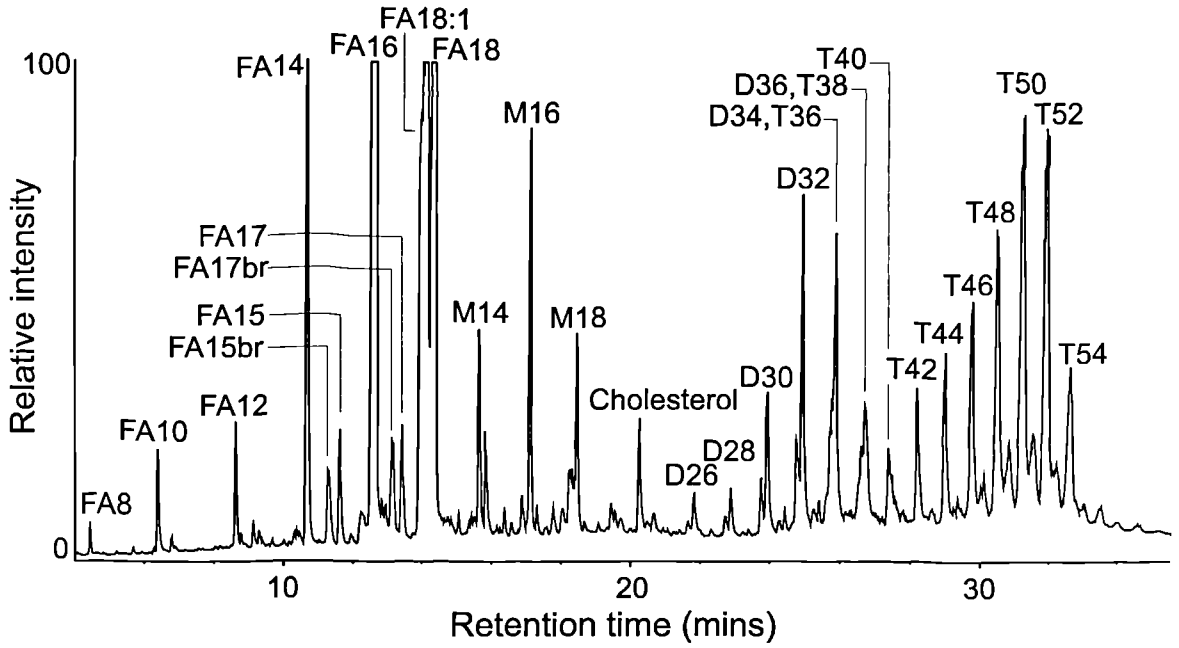


Figure 1.4 The HTGC profile of a trimethylsilylated TLE from a Late Saxon/early medieval 'Top Hat' vessel from West Cotton, Northamptonshire (sample WC30; Charters, 1996). Peak identities and GC conditions are as described in Figure 1.1.

The difficulty which has been encountered in identifying remnant milk fats is unfortunate since there is no other means of detecting dairying at archaeological sites. Evidence of dairying in the prehistoric period in Britain is limited solely to secondary evidence, for example, vessels associated with the procurement and utilisation of dairy products, such as putative ceramic 'cheese strainers' (Sherratt, 1981; Barker, 1981) and evidence from faunal studies, which have suggested that a high neonatal cull and a bias in the adult cull in domestic ruminant animals may indicate dairying (Legge, 1981; Crabtree, 1987). Since all these indicators remain unproven, a preferred approach to detecting dairying would be through the identification of the preserved residues of dairy products themselves.

1.5 The occurrence of lipids in nature

The reliable interpretation of data obtained by organic residue analysis is dependant upon a broad knowledge of the occurrence of different lipid moieties in nature and of their molecular structure. In general, long-chain esters or ethers of glycerol and derivatives, e.g. triacylglycerols (Figure 1.5) and phospholipids, are 'internal' lipids, serving a variety of structural and metabolic functions in living organisms, whereas waxes include internal and surface lipids, and comprise hydrocarbons, ketones, alkanes, acids, wax esters, alcohols, aldehydes and terpenoids (Kolattukudy, 1976).

Fatty acids are components of both fats and internal and surface waxes and occur in every living organism. They are the basic building blocks of adipose (storage) tissue in animals, which comprise principally $C_{16:0}$, $C_{18:0}$, $C_{18:1}$ and $C_{18:2}$ in terrestrial mammalian fats, while milk fats also contain diagnostic short-chain fatty acids ranging from $C_{4:0}$ to $C_{14:0}$. In fats from fresh water animals the component fatty acids are relatively rich in unsaturated C_{16} and C_{18} fatty acids with low contents of those of the C_{20} and C_{22} series; the unsaturated C_{16} fatty acids often forming 30% or more of the total fatty acids (Hilditch, 1956). Plant oils are characterised by a high abundance of $C_{16:0}$ together with a low abundance of $C_{18:0}$ (Mills and White, 1994), although surprisingly, there have been few reports of such commodities associated with archaeological ceramics. There is biological preference for the *cis* configuration at the C=C bonds [although some clay-catalysed isomerism to the *trans* form is known to occur in sediments (Killops and Killops, 1993)]. Oleic acid (*z*-9-octadecenoic

acid; referred to as Δ^9 $C_{18:1}$ in this thesis) is the most commonly occurring $C_{18:1}$ fatty acid found in animals, higher plants and algae, and *cis*-vaccenic acid (Δ^{11}) is particularly abundant in bacteria. However, these are by no means the only positional isomers occurring in nature. Figures 1.6(a) to (e) illustrate the structures of fatty acids occurring commonly in nature.

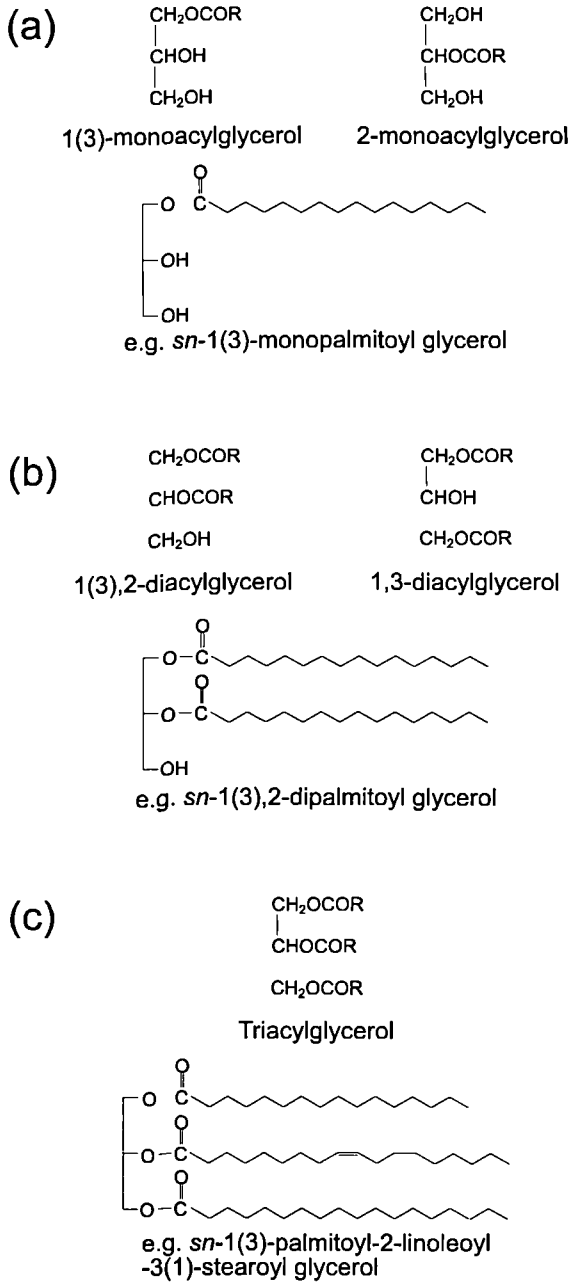


Figure 1.5 Structures of commonly occurring acyl lipids, including: (a) monoacylglycerols; (b) diacylglycerols, and (c) triacylglycerol (identified in olive oil and soybean oil by Mottram *et al.*, 1997).

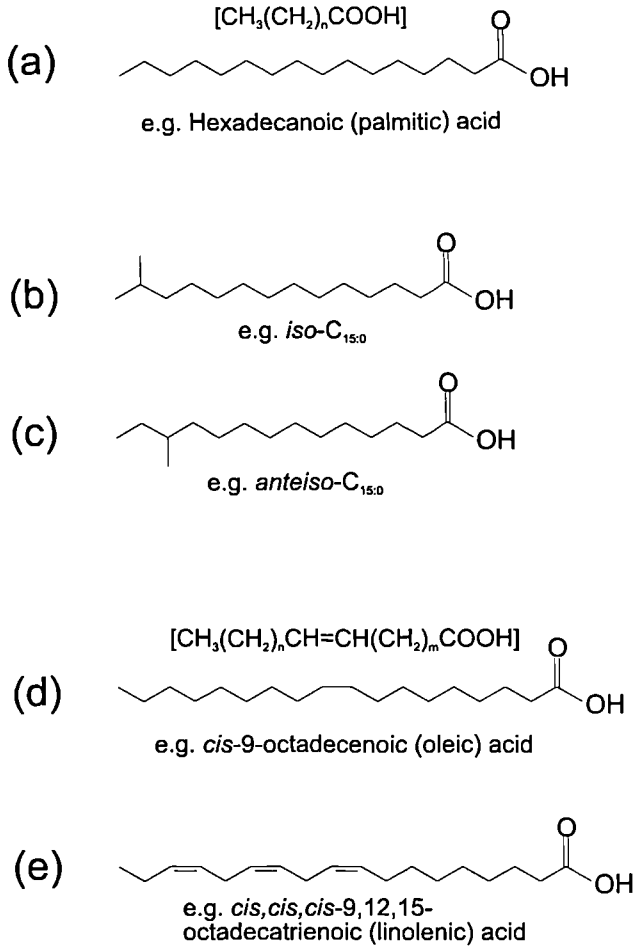


Figure 1.6 *Structures of commonly occurring fatty* carboxylic acids, including: (a) saturated straight-chain carboxylic acids; saturated branched-chain carboxylic acids in (b) *iso*- and (c) *anteiso*-forms; (d) monounsaturated carboxylic acids and (e) polyunsaturated carboxylic acids.

Sterols occur in low abundance in both vegetable oils and fats and their occurrence enables the distinction of vegetable oil from animal and fish oils due to the relatively low abundance of cholesterol in plant oils. Cholesterol [Fig. 1.7 (a)] is the major sterol (95-99%) in fish oils (Feely *et al.*, 1972; Kritchevsky *et al.*, 1967) and animal fats (Enser, 1991; Gunstone *et al.*, 1986). Eggs also contain large amounts of cholesterol (Mills and White, 1994). The principal sterol in plants is usually sitosterol [Fig. 1.7 (b)]; campesterol, stigmasterol and cholesterol also occur but in lesser abundances (Mudd, 1980). Ergosterol [Fig. 1.7 (c)] is the most common and most abundant sterol in eukaryotic microorganisms (algae, yeasts and moulds; Weete, 1980) but over 80 sterols have been detected in various

microorganisms. The occurrence of sterols in microorganisms and bacteria is given in Ratledge and Wilkinson (1988).

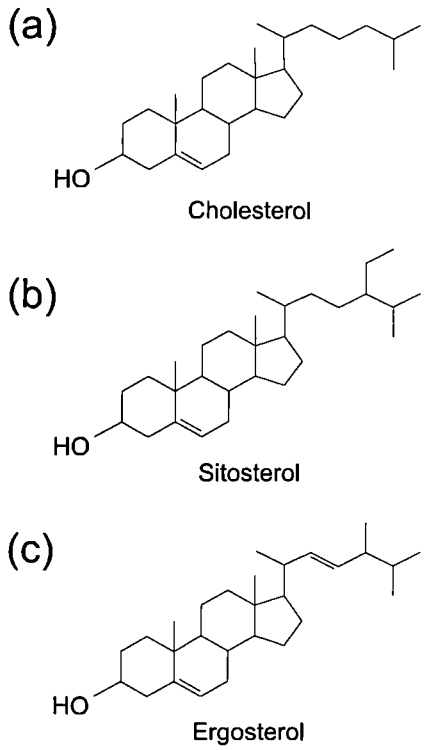


Figure 1.7 Structures of selected sterols, including: (a) cholesterol; (b) sitosterol, and (c) ergosterol.

Waxes occur widely amongst living organisms, with hydrocarbons, primary alcohols and primary alcohol wax esters ubiquitous in bacteria, fungi, algae, higher plants, insects and higher animals, including mammals. Ketones are restricted to higher plants and bacteria, whilst terpenoids are found widely in plants and animals and aldehydes are mainly found in higher plants. Figure 1.8 illustrates the structures of components commonly occurring in the waxes of living organisms.

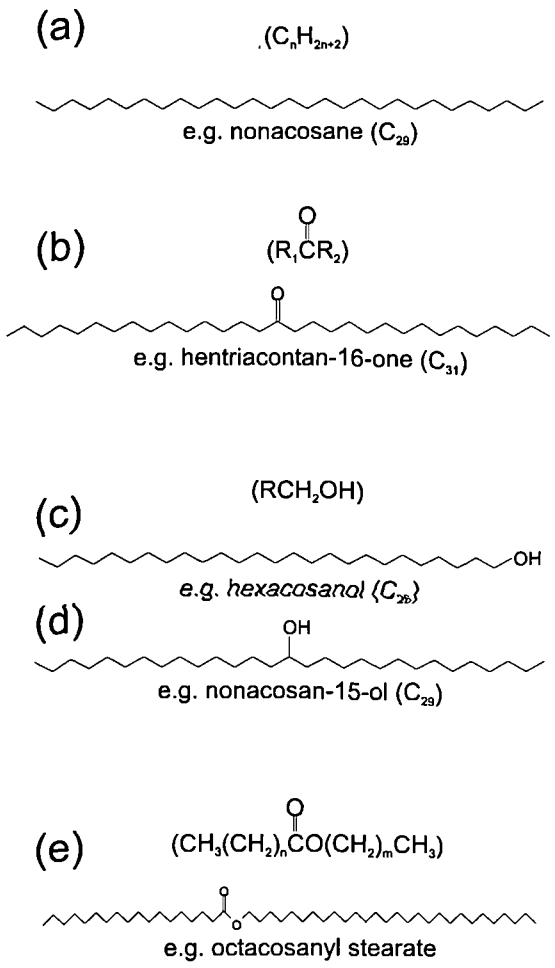


Figure 1.8 Structures of commonly occurring components of waxes, including: (a) saturated *n*-alkanes; (b) mid-chain ketones; (c) and (d) long-chain primary and secondary alcohols, respectively, and (e) wax esters.

1.6 The formation of animal fats

1.6.1 Fat composition

Most natural fats are a complex mixture of simple and mixed triacylglycerols, containing a variety of fatty acids, varying in chain lengths and degree of unsaturation, the most abundant natural fatty acids being the *cis*-configured mono- or polyunsaturated derivatives (Gurr and James, 1980). Other components include cholesterol, cholesteryl esters and phospholipids (Hilditch, 1956). In the triacylglycerol, three fatty acids are chemically bonded by an ester linkage to a glycerol backbone (Fig. 1.5) which can be readily broken by chemical or enzymatic hydrolysis.

Triacylglycerols normally comprise even numbers of carbon atoms in fatty acids due to their synthesis from acetic acid units. These units come from acetyl CoA, formed in glycolysis or by oxidation of fatty acids, from acetic acid and Coenzyme A. Fatty acids which comprise depot fats can be derived from a variety of sources and the situation is far from simple, with depot fats believed to be in a state of continual modification, e.g. from: (i) alteration by partial or complete hydrogenation of dietary fatty acids in the rumen (Katz and Keeney, 1966); (ii) synthesis *de novo* by rumen bacteria or by oxidation of other dietary components; (iii) modification of absorbed fatty acids by α or β-oxidation; (iv) desaturation or by chain elongation (Christie, 1978), and (v) *de novo* synthesis within the adipose tissue itself.

1.6.2 Precursors of fatty acid biosynthesis

Acetate is the major precursor and the adipose tissues are the major sites for endogenous fatty acid biosynthesis in the ruminant (Hanson and Ballard, 1967; Hood, *et al.*, 1972; Ingle *et al.*, 1972a). The fat content of the diets of the major domesticated animals is so low (<5%) that the major portion of the fat deposited as adipose fat is biosynthesised from acetate by the animal itself (Emery, 1980). Glucose also plays an essential role in fat synthesis: first it forms α-glycerophosphate, which is the precursor of glycerol to which fatty acids are esterified for triacylglycerol storage; secondly, glucose furnishes NADPH *via* the pentose pathway [NADPH is specifically required as a reducing agent at recurring steps in the synthesis of fatty acids (Swenson and Reece, 1993)]. Acetate has been shown to be the principal precursor for fatty acid synthesis in adipose tissue from sheep (Ballard *et al.*, 1972; Ingle *et al.*, 1972a; Vernon, 1976), cattle (Ingle *et al.*, 1972b; Yang and Baldwin, 1973) and goats (Škarda and Bartoš, 1969). Most of these studies showed that acetate carbon was incorporated into fatty acids between 10 and 100 times more rapidly than glucose carbon. Labelling experiments have been carried out to estimate the contribution of different carbon sources in *de novo* fatty acid synthesis. For example, rates of incorporation of ^{14}C from [1-^{14}C]acetate and of ^{3}H from $^{3}H_2O$ into fatty acids in sheep adipose tissue slices have been measured; the incorporation of ^{3}H from $^{3}H_2O$ into fatty acids is thought to be a measure of the total rate of fatty acid synthesis from all precursors (Jungas, 1968). The study concluded that acetate was the precursor for virtually all fatty acid synthesis in both perirenal and adipose tissue from sheep (Vernon, 1976). The relative

rates of incorporation of glucose and acetate carbon into fatty acids synthesised in adipose tissue in different species are given in Table 1.2.

Table 1.2 Relative rates of incorporation of acetate and glucose carbon during fatty acid synthesis [modified from Vernon (1980)].

Species	Relative rates of incorporation	Reference
Ruminants	Acetate >> glucose	Ingle *et al.* (1972b)
Rabbit	Acetate >> glucose	Smith (1975)
Guinea pig	Acetate > glucose	Saggerson (1974)
Pig	Acetate ≈ glucose	O'Hea and Leveille (1969b)
Rat	Acetate < glucose	Ballard *et al.* (1969)
Man	Mainly Glucose	Rook and Thomas (1983)

>> = 10 fold or greater than

1.6.3 *De novo* synthesis of lipid

In the *de novo* synthesis of palmitate (the major fatty acid produced) from acetyl CoA (Swenson and Reece, 1993; Garton, 1963; Morrison and Boyd, 1987), carboxylation (shown in reaction scheme **1**) of acetyl CoA to form malonyl CoA (*via* combination with the prosthetic group of acetyl CoA carboxylase and subsequent transferral to acetyl CoA) is the initial and rate limiting reaction and is catalysed by acetyl CoA carboxylase. In the remaining steps, acetic and malonic acids react as thiol esters of acyl carrier protein (ACP; **2** and **3**).

(1) $CH_3CO–S–CoA + \mathbf{CO_2} + ATP \rightleftharpoons \mathbf{HOOC}–CH_2CO–S–CoA + ADP + phosphate$
Acetyl CoA — Malonyl CoA

(2) $CH_3CO–\mathbf{S–CoA} + ACP–SH \rightleftharpoons CH_3CO–\mathbf{S–ACP} + CoA–SH$
Acetyl–S–ACP

(3) $HOOCCH_2CO–\mathbf{S–CoA} + ACP–SH \rightleftharpoons HOOCCH_2CO–\mathbf{S–ACP} + CoA–SH$
Malonyl–S–ACP

Next, acetyl–S–ACP condenses (**4**) with malonyl–S–ACP to give the acetoacetyl moiety, a four-carbon chain, and the decarboxylation regenerates the CO_2 taken up in reaction **1**.

(4) $\mathbf{CH_3CO}$–S–ACP + $HOOCCH_2CO$–S–ACP $\rightleftharpoons$
$\mathbf{CH_3CO}$–CH_2CO–S–ACP+ $\mathbf{CO_2}$ + ACP–SH
Acetoacetyl–S–ACP

The subsequent steps involve reduction to an alcohol (**5**), dehydration (**6**) and hydrogenation (**7**) to generate a butyryl group which is transferred to the peripheral thiol of fatty acid synthetase. Each subsequent elongation sequence includes binding of another malonyl CoA to the central thiol of the ACP, condensation with the growing saturated fatty acid, reduction, dehydration and hydrogenation. These steps are repeated seven times until palmityl-fatty acid synthetase is formed. The reducing agent for both **5** and **7** is reduced nicotinamide adenine dinucleotide phosphate (NADPH)

(5) $\mathbf{CH_3CO}$–CH_2CO–S–ACP + NADPH+ H^+ $\rightleftharpoons$ $\mathbf{CH_3CHOH}$–CH_2CO–S–ACP + $NADP^+$
β-hydroxybutyryl–S–ACP

(6) $\mathbf{CH_3CHOH}$–CH_2CO–S–ACP $\rightleftharpoons$ $\mathbf{CH_3CH}$=CHCO–S–ACP + H_2O
Crotonyl–S–ACP

(7) $\mathbf{CH_3CH}$=CHCO–S–ACP + NADPH + H^+ $\rightleftharpoons$ $\mathbf{CH_3CH_2}$–$\mathbf{CH_2CO}$–S–ACP + $NADP^+$
n-Butyryl–S–ACP

1.6.4 Products of fatty acid synthesis

The principal fatty acids synthesised from acetate in adipose tissue slices from cattle (Pothoven *et al.*, 1974) and sheep (Deeth and Christie, 1979) were long-chain fatty acids, namely, $C_{16:0}$, $C_{18:0}$ and $C_{18:1}$. Small amounts of $C_{14:0}$ and $C_{16:1}$ were also formed, but in general, negligible amounts of short-chain fatty acids were detected (Pothoven *et al.*, 1974). However, in ruminant milk fat, numerous medium- and short-chain fatty acids have been identified (Smith *et al.*, 1968; Watts and Dils, 1968). These are synthesised *de novo* within the mammary gland. Medium-chain fatty acids appear to be incorporated directly into the milk triacylglycerols and are neither desaturated nor elongated. A chain-terminating enzyme has been found in the mammary gland of non-ruminant animals which controls the production of medium-chain fatty acids. This medium-chain acylthioester hydrolase has been isolated from the cytosol of lactating rabbit (Knudsen *et al.*, 1976) and rat (Smith, 1980) mammary gland. When added to purified fatty acid synthetase (in the presence of rate-limiting amounts of malonyl CoA) the pattern of fatty acids synthesised

changes to predominantly $C_{8:0}$ and $C_{10:0}$ acids (Knudsen *et al.*, 1976). Immunochemical techniques have shown that the appearance of this enzyme in rabbit mammary gland coincides with the onset of milk fat synthesis (Chivers *et al.*, 1977). Furthermore, only non-ruminant tissues which contain the acylthioester hydrolase are able to synthesise medium-chain fatty acids (Smith, 1980). However, no equivalent medium-chain hydrolase could be detected in studies of the cytosol of lactating goat mammary gland (Grunnet and Knudsen, 1979a). The synthesis of medium-chain fatty acids from purified fatty acid synthetases from lactating ruminants was found only to occur in the presence of an unidentified microsonal factor obtained from lactating ruminant or non-ruminant mammary gland (Grunnet and Knudsen, 1979b), suggesting that the medium-chain acylthioester involved in fatty acid synthesis in the ruminant is an inherent component of the fatty acid synthetase complex in the tissue. Butyryl-CoA and hexanoyl-CoA (derived from 3-hydroxybutyrate from the bloodstream or from acetyl-CoA by fatty acid synthetase) are known to be esterified into triacylglycerols *in vivo* in cow mammary gland by microsomal diacylglycerol acyltransferase (Hansen and Knudsen, 1980). The mechanism for the coupling of these short-chain acyl-CoA esters in triacylglycerol synthesis is not known but is not attributed to any special chain length specificity of the microsomal diacylglycerol acyltransferase in this tissue for these short-chain acyl-CoA esters (Marshall and Knudsen, 1979).

1.6.5 Triacylglycerol synthesis

The major lipid in the diet of non-ruminant animals is triacylglycerol, which is hydrolysed to free fatty acids and monoglyceride in the small intestine. The fatty acids are resynthesised into triacylglycerols by two pathways: the monoglyceride and the phosphatidate pathways. Due to the large amounts of monoglycerides absorbed, most of the resynthesis (70-80%) is *via* monoglyceride (Mattson and Volpenhein, 1964; Kayden *et al.*, 1967). In ruminants, food is subjected to microbial fermentation in the rumen, where cellulose and carbohydrates are converted to volatile acids, particularly acetate (Elsden, 1946), proteins are broken down to amino-acid and ammonia, and lipids are hydrolysed prior to biohydrogenation. The digesta lipid passing to the small intestine in ruminant animals is already in the form of (predominantly saturated) free fatty acids. In this case the major pathway of resynthesis is *via* the phosphatidate pathway. Thus, in non-ruminants the

fatty acid composition of lymph triacylglycerols will resemble that of the diet, but in ruminants hydrogenation occurring in the rumen results in large amounts of $C_{18:0}$ being incorporated into lymph triacylglycerols (Harrison and Leat, 1975). In the phosphatidate pathway fatty acyl CoA is essential in the formation of glyceryl ester linkages (Fig. 1.9). The first stage involves acylation of free alcohol groups of the glycerol-3-phosphate by two molecules of fatty acyl-CoA (in the presence of glycerol-3-phosphate acyl-transferase) to yield a phosphatidic acid. The phosphatidic acid is hydrolysed to give a diacylglycerol which reacts with a third fatty acyl-CoA to give a triacylglycerol (McDonald, 1988).

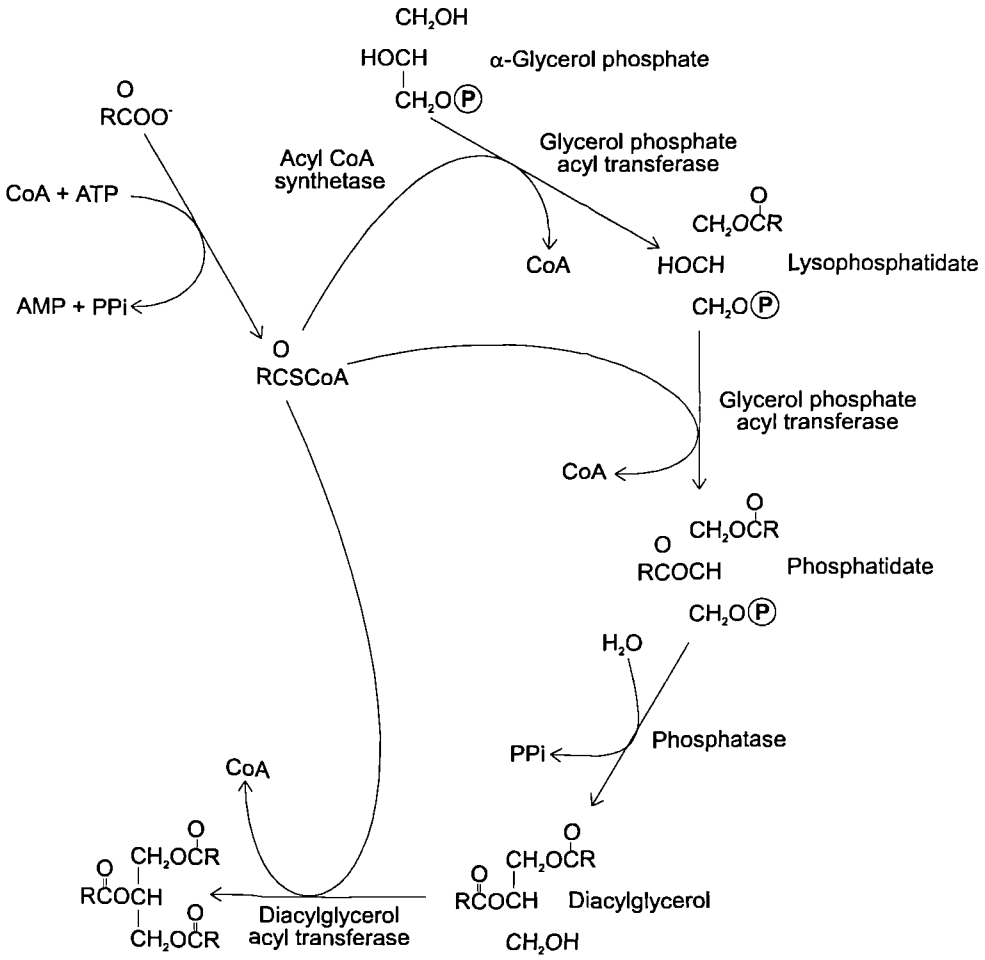


Figure 1.9 Biosynthesis of triacylglycerols by way of the phosphatidate pathway (Swenson and Reece, 1993).

Rat feeding experiments using triacylglycerols in which the fatty acids occupied specific positions of the glyceride molecule have enabled the elucidation of the digestion and absorption pathway in non-ruminants (Mattson and Volpenhein, 1964). In the intestinal

wall the *sn*-1 and -3 position fatty acids are hydrolysed in preference to the *sn*-2 position fatty acids due to enzyme specificity (Mattson and Beck, 1955; Hoffman and Borgström, 1963) and because of their transfer to a water-soluble phase (Hoffman and Borgström, 1962, 1963). The study indicated that approximately 75% of the glycerol of dietary triacylglycerol is absorbed as monoacylglycerol and 75% of the fatty acids of dietary triacylglycerol are absorbed as free acids. The *sn*-2 monoacylglycerols can be acylated directly to triacylglycerol in the intestinal wall without passing through a phosphorylated intermediate (Clark and Hubscher, 1960; Senior and Isselbacher, 1962; Johnston and Brown, 1962). The *sn*-2-monoglyceride pathway of fat absorption has also been found to be the major route of fat absorption for man during normal digestion and absorption of dietary triacylglycerol (Kayden *et al.*, 1967). Senior and Isselbacher (1962) have suggested that an enzyme system catalyses the direct acylation of monoacylglycerols to diacylglycerols by condensation of fatty acyl-CoA derivatives with monoacylglycerols (Fig. 1.10). The initial activation of free fatty acids to fatty acyl-CoA by a long-chain fatty acid thiokinase was found to be necessary for the resynthesis of higher glycerides from free fatty acids.

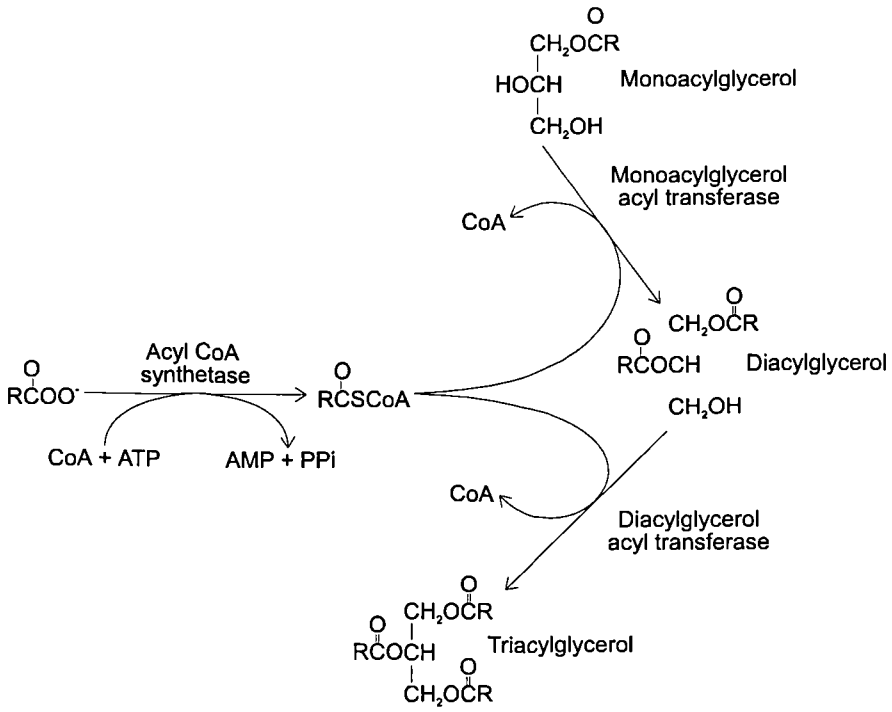


Figure 1.10 Biosynthesis of triacylglycerols by way of the monoacylglycerol pathway (Swenson and Reece, 1993).

1.6.6 Incorporation of dietary fatty acids

In adipose tissue the adipocyte plasma membrane is freely permeable to fatty acids. In ruminants the mixture of adsorbed fatty acids is particularly rich in $C_{18:0}$ formed as a result of ruminal hydrogenation of $C_{18:1}$, $C_{18:2}$ and $C_{18:3}$ components. The efficiency of adsorption of fatty acids is in the order $C_{18:0}<C_{16:0}<C_{18:1}$ in ruminants as in all animals (Harrison and Leat, 1975), but nevertheless $C_{18:0}$ is still particularly well adsorbed in ruminants. In non-ruminants, e.g. pigs, a greater proportion of the precursors for adipose fat formation are pre-formed in the micelles which permeate the adipocyte plasma membrane. Thus, the fatty acid content of the adipose tissue much more closely reflects the composition of the dietary fat.

Depot fats in different animal species incorporate dietary fatty acid components to different extents, for example, monogastric grazing animals, incorporate substantial proportions of dietary fatty acids like $C_{18:2}$ and $C_{18:3}$ acids directly into their depot fats (Payne, 1971). This is also the case for omnivores, e.g. humans (Shorland *et al.*, 1969) and pigs (Christie *et al.*, 1972), which accumulate $C_{18:2}$ into their adipose tissue. In comparison, the depot fats of sheep and deer (ruminants) feeding in the same field contain only trace amounts of these fatty acids (Shorland, 1953; Shorland *et al.*, 1952; Brooker and Shorland, 1950). The latter can modify plant polyunsaturated fatty acids by extensive microbial breakdown processes in the rumen. Labelling experiments have shown dietary triacylglycerols in human milk fat are incorporated unaltered (Hachey *et al.*, 1984). Numerous studies on the dietary contribution to ruminant milk fats have been conducted and are discussed in Section 1.8.5.

1.6.7 Positional distribution of fatty acids

During the processes of lipid digestion, absorption, transport and utilisation by tissues, triacylglycerols undergo hydrolysis and re-esterification both at the intestinal level and at the site of utilisation (liver, muscle, adipose tissue, etc.) and this results in the occurrence of triacylglycerols with characteristic configurations of fatty acyl moieties on the glycerol backbone, partly due to an enzyme specificity associated with their biosynthesis (Coleman, 1965; Entressangles *et al.*, 1966). The central atom of the triacylglycerol is chiral, so the three positions on the glycerol backbone of the molecule are chemically distinct. The

positions are numbered according to the stereospecific numbering notation proposed by Hirschmann (1960; Fig. 1.11).

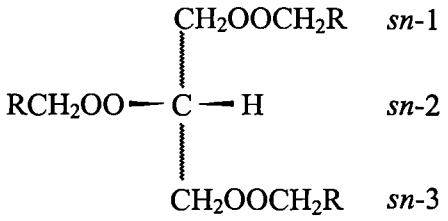


Figure 1.11 Fischer projection of a triacylglycerol molecule.

For the majority of the natural triacylglycerols, fatty acids are located at specific positions on the glyceride backbone, generally occurring with the shorter and more unsaturated acids predominating at the 2-position (except in pigs and peccaries) and the saturated acids at the 1- and 3-positions (Table 1.3; Anderson *et al.*, 1970b; Brockerhoff *et al.*, 1966; Christie and Moore, 1969; Christie and Moore, 1971; Kagawa *et al.*, 1996).

Mammalian milk fats, with the exception of echidna (Parodi, 1982), comprise butyric ($C_{4:0}$) and caproic ($C_{6:0}$) acids at the *sn*-3-position, with other acids such as $C_{18:0}$ which is found at the *sn*-1-position, also distributed preferentially (Kuksis *et al.*, 1973; Pitas *et al.*, 1967). In lamb (Christie and Moore, 1971) and beef (Brockerhoff *et al.*, 1966) fat $C_{16:0}$ is predominantly found in position *sn*-1, with smaller amounts in *sn*-2 and -3, and with $C_{18:0}$ occurring mainly in positions *sn*-1 and -3. The $C_{18:1}$ fatty acid is located mainly in position *sn*-2. Ruminant depot fats will contain a proportion of odd-carbon number and branched-chain fatty acids produced by rumen microflora, as discussed in Section 1.8.2. The difference between lamb and beef fat is that the proportion of $C_{18:0}$ is highest at the *sn*-3-position in beef fat but dominates at the *sn*-2-position in lamb fat (Brockerhoff *et al.*, 1966). In pork fat $C_{16:0}$ is predominant at the *sn*-2-position with the $C_{18:1}$ fatty acid mainly at the *sn*-1 and *sn*-3-positions (Christie and Moore, 1970). Due to the fact that pig fat reflects the diet of the animal much more closely than ruminant fat, there is a higher proportion of unsaturated moieties in pig triacylglycerol. The *sn*-1 and -3 positions of chicken fat are comprised mainly of $C_{16:0}$ and $C_{18:1}$, with the latter predominating at *sn*-2 (Brockerhoff *et al.*, 1966). Chicken fat is unusual in that the fatty acid distribution in the *sn*-1 and *sn*-3 positions are very similar.

Table 1.3 Stereospecific distributions of fatty acids in depot fat triacylglycerols of animal fats (mol %).

Animal fat	*sn*-position	Saturated fatty acid			Unsaturated fatty acid			
		$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{16:1}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
Lamb [a]	1	1.0	35.1	47.0	1.6	4.4	-	-
	2	3.8	13.6	15.2	2.2	51.7	5.1	-
	3	2.7	15.5	41.9	1.0	25.5	2.1	-
Beef [b]	1	4.0	41.0	17.0	6.0	20.0	4.0	<1.0
	2	9.0	17.0	9.0	6.0	41.0	5.0	<1.0
	3	1.0	22.0	24.0	6.0	37.0	5.0	<1.0
Pork [c]	1	0.9	9.5	29.5	2.4	51.3	6.4	-
	2	4.1	72.3	2.1	4.8	13.4	3.3	-
	3	<0.2	0.4	7.4	1.5	72.7	18.2	-
Chicken [b]	1	2.0	25.0	6.0	12.0	33.0	14.0	2.0
	2	1.0	15.0	4.0	7.0	45.0	23.0	3.0
	3	1.0	24.0	6.0	12.0	35.0	14.0	3.0
Man [b]	1	4.0	39.0	10.0	5.0	33.0	3.0	1.0
	2	11.0	10.0	2.0	11.0	50.0	9.0	1.0
	3	1.0	25.0	9.0	4.0	51.0	5.0	1.0
Horse [b]	1	3.0	39.0	6.0	7.0	27.0	5.0	11.0
	2	7.0	9.0	1.0	10.0	29.0	17.0	25.0
	3	3.0	30.0	7.0	6.0	37.0	5.0	11.0
Fish (Tuna)[d]	1	3.9	35.8	12.2	7.3	16.5	1.6	0.02
	2	5.2	21.3	2.5	3.9	6.17	1.3	0.2
	3	2.2	10.4	2.8	4.1	18.5	1.9	0.2

[a] Christie and Moore (1971)
[b] Brockerhoff *et al.* (1966)
[c] Christie and Moore (1970)
[d] Myher *et al.* (1996)

The use of these characteristics of fatty acid distributions in animal fat triacylglycerols in determining species origins has been explored in our laboratory by Crossman (1998). HPLC-APCI/MS analysis enabled the identification of the positional distribution of fatty acids in triacylglycerols in ruminant and non-ruminant fresh and archaeological fats. APCI typically gives spectra for triacylglycerols which show the protonated molecular ion, $[M+H]^+$, diacylglycerol ions, $[M-RCO_2]^+$ and acylium ions, RCO^+. The positional distributions of fatty acids can be identified by the abundance of the diacylglycerol ions, since the least abundant diacylglycerol ion is the one formed by removal of the *sn*-2-position fatty acid as this is energetically less favourable than the *sn*-1 or *sn*-3-positions (Mottram and Evershed, 1996).

Overall, the study supported the origins previously assigned to the archaeological fats by comparison of the ratio of $C_{16:0}$ and $C_{18:0}$ in the *sn*-2-position of triacylglycerols. The *sn*-2-position in fresh ruminant fats contains a $C_{16:0}$:$C_{18:0}$ ratio of approximately 60:40, whereas fresh fats from non-ruminants (e.g. pig) typically contain a ratio of 95:5 (Rossell, 1991; Table 1.4). As discussed earlier (Section 1.6.5), triacylglycerols in non-ruminant fats are stereospecifically cleaved by pancreatic lipase to yield *sn*-2-monoacylglycerols which are used in the resynthesis of triacylglycerols in body tissues. Subsequently, the distribution of fatty acids in the *sn*-2-position of non-ruminant triacylglycerols will reflect that of the diet. The data obtained for the archaeological fats compared very well with that obtained for the reference fats. Furthermore, comparison of enzymatic cleavage and HPLC-APCI/MS techniques for determining the fatty acids at the *sn*-2-position, has shown the techniques to be reliable and the data obtained comparable. The study indicated the potential of this chemical parameter in determining either a ruminant or non-ruminant origin for remnant animal fats.

Table 1.4 Ratio of $C_{16:0}$ to $C_{18:0}$ in ruminant and non-ruminant depot triacylglycerols.

Sample	Overall $C_{16:0}$:$C_{18:0}$ ratio		*sn*-2-position $C_{16:0}$:$C_{18:0}$ ratio	
	Ruminant	Non-ruminant	Ruminant	Non-ruminant
Fresh fat	44:56	60:40	41:59	86:14
Archaeological fat	67:33	41:58	45:55	81:19

1.7 The formation of ruminant milk fat

Milk lipids consist primarily of triacylglycerols (97 to 98% by wt; Dimick *et al.*, 1970), although there are also very small amounts of phospholipids, cholesterol, fat-soluble vitamins, squalene, free fatty acids and monoglycerides (Garton, 1963; Jack and Smith, 1956). Milk fat from herbivores contains relatively high abundances of fatty acids with carbon chain lengths from 4 to 14. Labelling experiments (Barcroft *et al.*, 1944; Popjak *et al.*, 1951) have suggested that the high proportion these short-chain components may derive from the bacterial fermentation of carbohydrates in the alimentary canal, i.e. mainly acetic and propionic, with absorption occurring directly from the ruminant stomach.

It is now well recognised that milk fats may come: (i) directly from absorbed dietary fat; (ii) from fatty acids synthesised in the mammary gland, or (iii) from the retrieval of adipose tissue fat, and reflects some combination of two or more of these sources (Popjàk *et al.*, 1951). Studies on the incorporation of labelled plasma lipids have shown that about 50% of the milk lipids (especially the long-chain fatty acids) are derived from plasma and 50% are synthesised by the mammary gland (Riis *et al.*, 1960; Riis and Moustgaard, 1962). A large part if not all of the plasma lipids are believed to be derived from the chylomicron and low-density lipoproteins through hydrolysis by lipoprotein lipase, absorption by the mammary gland and reassembly into milk triacylglycerols (McCarthy *et al.*, 1960). Studies by Duncan and Garton suggested that the increase observed in plasma lipid concentration associated with lactation reflects demands by the udder for the plasma lipids needed in milk fat synthesis.

Fatty acids from C_4 to C_{16} are synthesised largely in the mammary gland, starting with either acetate or β-hydroxybutyrate and proceeding by stepwise condensation with acetyl-CoA units to form the longer-chain acids (Palmquist *et al.*, 1969). $C_{16:0}$ can come either from mammary synthesis or the blood, whereas all C_{18} and longer-chain components are derived from the blood but may be of dietary or adipose origin. Dietary fats are transferred efficiently to milk fat, especially when adipose tissue storage is not extensive (Mattos and Palmquist, 1977). Several studies indicate transfer efficiencies of 50-75% for dietary long-chain fat to milk (e.g. Bitman *et al.*, 1973; Cook *et al.*, 1972), but proportions reflect diet, supply and metabolic priorities and change is dependant on stage of production and level of lactation. The influence of dietary fat on milk fat composition is extremely complex (Storry, 1981), with net transfer of acids from the diet to milk reported for the following acids:

$<C_{12:0}$	low transfer
$C_{12:0}$	27%
$C_{16:0}$	29-90%
C_{18} (total)	19-65%
C_{20}-C_{22} (total)	low transfer

In general, there is a low transfer of components of chain length $<C_{12}$ and $>C_{20}$, and a relatively high net transfer of the C_{16} and C_{18} components directly from the diet to milk fat.

As a result of studies of energy balance in cows (Broster, 1972; Swan, 1976) and sheep (Robinson, 1973), it has been suggested that the lactating ruminant is in a state of positive energy balance for most of the pregnancy and would thus accumulate lipid (energy) reserves, however, in late pregnancy the demands of the foetus(es) and milk production may result in a period of negative energy balance and mobilisation of adipose tissue lipid (Vernon, 1980). Studies have also indicated that high milk yielding cows will divert a greater proportion of their dietary energy into milk production (Broster, 1972; Swan, 1976), rather than low-yielding animals which divert a greater proportion into the accumulation of body tissue.

1.8 Factors affecting adipose fat composition in different animal species

1.8.1 Mechanisms of digestion

Detailed descriptions of physical characteristics and gastric movements in the stomachs of ruminant and non-ruminant animals are given by Frandson and Spurgeon (1992) and Swenson and Reece (1993). The distinctions described between animals with simple or complex stomachs are augmented by the differences in digestive mechanisms between herbivorous and carnivorous animals.

In herbivores, foodstuffs are broken down in the digestive tract by the growth and development of micro-organisms. Ruminants have a large forestomach which constitutes a fermentation vat where the food material is attacked by micro-organisms, although enzymatic digestion does occur following microbial breakdown and the microbial bodies themselves are eventually digested by the animal. In non-ruminant herbivores the cecum and colon are large, well-developed organs where microbial digestion takes place; fermentation digestion follows enzymatic digestion so that only the fermentation products and not the bacterial bodies are available for digestion and absorption by the host. Microbial digestion is of little importance in carnivores because the digestive processes are virtually complete in the small intestine. The colon is short and nonsacculate and the cecum is relatively undeveloped (Swenson and Reece, 1993). Microbial and enzymic digestion differ significantly in that fibrous carbohydrate sources that have the β-linked glucose polymers and are not broken down by mammalian enzymes are readily attacked by

microbial enzymes (Swenson and Reece, 1993), thus allowing cellulose, the most abundant carbohydrate form present in the plant to become a major nutrient for ungulates. In non-leguminous plants, carbohydrates make up about 66% of the dry matter, both in roughages (straw and grasses) and in grains (Czerkawski, 1986). Lipids comprise the cell membranes, making up between 3-10% of dry matter. Fewer than 50% of the total lipids are free fatty acids (the majority are phospholipids), with $C_{16:0}$, $C_{18:2}$ and $C_{18:3}$ acids predominating.

1.8.2 Biohydrogenation

It is well established that esterified lipids from plant material are hydrolysed in the rumen by microbial action (Garton *et al.*, 1958; 1961), before hydrogenation of the resultant free, unsaturated fatty acids. $C_{18:1}$, $C_{18:2}$ and $C_{18:3}$ fatty acids from dietary forage have *cis* double bonds, however bacterial hydrogenation of these unsaturated fatty acids in the rumen of sheep and cows leads to the formation of *trans*-configured acids, which are particularly high in lamb fat (Whitehead and Turrel, 1988). In studies of the action of intestinal microflora, Eyssen and Parmentier (1974) recognised that the first step in the biohydrogenation of $C_{18:2}$ is in fact a shift of one of the double bonds in the *cis* fatty acid, resulting in a conjugated $\Delta^{9, 11}$ or $\Delta^{10, 12}$ *trans*-isomer, indicating that biohydrogenation does not simply involve successive additions of hydrogen across a double bond (Wilde and Dawson, 1966). Monogastric animals, e.g. pigs, cannot modify unsaturated fatty acids in this way so *trans* acids tend to be lower in porcine depot fats, the only possible source of these acids being the diet of the animal (Duncan and Garton, 1967).

1.8.3 End products of ruminant digestion

Due to the distinctive nature of the ruminant stomach, the fermentation end products [volatile fatty acids (VFAs), etc.] that the ruminant absorbs and uses as its prime metabolic substrates are quite different from the end products of digestion (glucose, etc.) in non-ruminants. The main fermentative end products of all carbohydrates are acetic, propionic and butyric acids. The fermentation of protein yields these acids together with valeric acid (C_5) and the branched VFAs, isobutyric and isovaleric acid. VFAs enter the circulatory system as acetate, glucose (from propionate) and β-hydroxybutyrate (from butyrate) which can be readily metabolised by most tissues of the body. β-hydroxybutyrate is used to provide the first four carbon units in the mammary synthesis of about half of the short- and

medium-chain fatty acids (C_4-C_{14}) characteristic of ruminant milk. Acetate, the most abundant VFA is the prime metabolic substrate, converted in the body tissues to acetyl CoA for use in the citric acid cycle. The hydrogenated long-chain fatty acids are absorbed and taken up by the adipose tissue and by the lactating mammary gland.

1.8.4 End products of non-ruminant digestion

While traces of disaccharides are absorbed from the small intestine, the bulk of dietary carbohydrate is absorbed as monosaccharide (glucose, galactose and fructose) in non-ruminant animals. Dietary fats, including triacylglycerols, phospholipids and sterol esters are either partially or wholly hydrolysed prior to absorption by the intestinal wall from a mixed micellular solution (Hofmann and Borgström, 1964; Johnston and Borgström, 1964). Dietary fat provides approximately 60% of the dietary energy and thus fats are efficiently absorbed, however, the position of the fatty acid in the glyceride molecule has been reported to affect its digestibility (Freeman *et al.*, 1968). The fatty acids and monoglycerides are re-esterified to triacylglycerols inside the epithelium. The triacylglycerols are then associated with cholesterol, cholesterol esters, phospholipids and a small amount of protein to form chylomicrons. Formation of the chylomicron (similar to the water-soluble micelle) facilitates the transport of water-insoluble triacylglycerol across the cell membrane (Swenson and Reece, 1993).

In the large intestine, both soluble (starch) and insoluble (fibre) carbohydrates are degraded by microbial enzymes, mainly to hexoses, although the fibrous portion of food is fairly indigestible to non-ruminant animals. These are metabolised by bacteria to VFAs and gases, the end products of carbohydrate fermentation. Only a small amount of lactate and succinate are produced under normal conditions; a high fibre diet will produce more acetate.

1.8.5 Diet

The effect of diet on the composition of ruminant fats has been widely investigated due to the economic importance of meat quality. The influence of dietary fat on the composition of depot fats varies in different animal species; where the rabbit and to a lesser extent the horse incorporate $C_{18:3}$ from the diet into their depot fats, the depot fats of sheep and deer

feeding in the same field contain only trace $C_{18:3}$ (Shorland, 1953; Shorland *et al.*, 1952; Brooker and Shorland, 1950).

The fatty acid composition of triacylglycerols in the adipose tissue of ruminants is remarkably non-responsive to dietary changes. Biohydrogenation of unsaturated fatty acids in the rumen is the major reason why the depot fats of ruminant animals are not adversely affected by elevated levels of unsaturated acids in the diet (Tove and Mochrie, 1963; Tove and Smith, 1960). Furthermore, when saturated fatty acids of varying chain length are fed, these acids are elongated in the adipose tissues (Hilditch, 1956). Cattle fed diets containing elevated levels of $C_{18:1}$ (from rapeseed) exhibited little effect from the diet due to apparent indigestibility of the rapeseed (St. John *et al.*, 1987).

Wild or free ranging ruminants do not show the same enhanced deposition of neutral fat seen in concentrate fed ruminants (Ledger, 1968). There tends to be a lesser proportion of neutral glycerides and a greater proportion of structural phospholipids in the carcass fat of wild ruminants. The changes due to a roughage diet are thought as much as anything to be a result of altered rumen fermentation. High forage diets are reported to increase the deposition of saturated acids, primarily $C_{16:0}$ (McDonald *et al.*, 1988) and to increase proportions of branched-chain and *trans*-unsaturated monoenoic acids, whilst proportions of $C_{18:2}$ are lowered (Marmer *et al.*, 1984).

Diet can have a potentially much more significant effect on the composition of omnivores, e.g. pork fat, than in ruminant animals. Dietary saturated and unsaturated fatty acids are deposited virtually unchanged in pig depot fats, amplified by the fact that pigs can digest much greater proportions of fat in the diet than ruminants (Busboom *et al.*, 1991; St. John *et al.*, 1987); little modification of the fats occurs unless utilised for energy (Leat *et al.*, 1964; Koch *et al.*, 1968). Labelling studies have shown dietary fatty acids are absorbed into the bloodstream and deposited into the adipose tissue, e.g. pigs fed $C_{18:2}$-enriched diets have been shown to incorporate $C_{18:2}$ into their adipose tissue triacylglycerols (Mills *et al.*, 1976). Less common fatty acids such as branched-chain components are also deposited in pig depot fats when present in the diet (Bastijns, 1970). The feeding of supplementary

dietary fat (olive oil) to broilers resulted in an increase in the ratio of monounsaturated to saturated fatty acids in breast and thigh meat (O'Neill *et al.*, 1998).

1.8.6 Age

The age of the animal has been reported as a major factor affecting fat composition, for example, the $C_{18:0}$ content of the adipose fat of cattle is known to decline markedly in older animals (Enser, 1991 and references therein). Subcutaneous fat becomes softer with age as a result of the decrease in the proportion of $C_{18:0}$ and increase in $C_{18:1}$ (Leat, 1975, 1977; Pothoven *et al.*, 1974; Pyle *et al.*, 1977). In young cattle, prior to the development of the rumen, dietary fatty acids are deposited directly in their adipose tissues. The desaturation activity of subcutaneous adipose fats also increases with age: Leat (1975) has shown that at 6 months old, the proportions of $C_{16:0}$ and $C_{18:0}$ were 21% and 15%, respectively (wt % of the total), compared with 29.5% and 6.3% at 15 months. The proportion of $C_{14:0}$ remained relatively low, between 2.3% and 2.9%. Effects of breed and sex on fatty acid composition are relatively insignificant, depending mainly on fatness (Sumida *et al.*, 1972; Gillis *et al.*, 1973).

1.8.7 Depot site

Fatty acid composition has been shown to vary with depot site, with marked differences between internal and subcutaneous fats. Sheep perirenal fat has been shown to contain higher proportions of $C_{18:0}$ and lesser amounts of $C_{18:1}$, whereas subcutaneous fats (chest or rump) contain a higher proportion of unsaturated fatty acids (Christie and Moore, 1971; Leat, 1977; Garcia *et al.*, 1979). $C_{18:0}$ in sheep fat has been shown to vary between 11% (rump) and 34% (perirenal) by weight at different tissue sites in sheep aged 4-5 years (Duncan and Garton, 1967). Results obtained for bovine tissues were similar to sheep in that internal fats had higher levels of saturated fatty acid components than subcutaneous fats (Hartman *et al.*, 1955). Pork fat shows less variation between cuts than beef fat (Whitehead and Turrel, 1988). The higher proportion of saturated fatty acids, especially $C_{18:0}$, comprising the triacylglycerols in internal fats (e.g. perirenal) gives it its hardness in order to protect the organs, while subcutaneous fat is softer, containing a lower proportion of saturates. Differences in composition between subcutaneous fats are reportedly small (Terrell *et al.*, 1967, 1969).

1.9 The stable carbon isotope composition of natural fats

Apart from analytical error, which in our laboratory is ± 0.3‰, it is widely recognised that 'ecological variability', diet and temperature-dependant isotope effects associated with fat synthesis are the major factors affecting the isotopic study of natural diets:

1.9.1 Ecological Variability

'Ecological variability' is the 0.2 to 2‰ standard deviation (which averages at about 0.6‰) found for animals of the same species raised in similar environments on a similar diet (DeNiro and Epstein, 1978; Teeri and Schoeller, 1979; Tieszen *et al.*, 1983). This variability is attributed to: (i) inhomogeneity in a single food source; (ii) selectivity; (iii) the biochemical state of the animal; (iv) intraorganism variation, and (v) seasonal differences.

In nature it is likely that there will be a certain amount of variation in the food source of grazing animals, however the effect of inhomogeneous food is counteracted by the fact that animal tissue represents an average of food assimilated over time (Gearing, 1994). Certain species are known to be more selective feeders than others and some may have a greater ability to digest certain foodstuffs than others which may bias the isotopic composition of the ingested material. Gearing (1994) suggests that there may be a natural error of up to 2‰, determined by the size of animal, its health and age. Other reports suggest that size sometimes has an effect, but not always (Fry and Parker, 1979; Gearing *et al.*, 1984). No differences are found with sex (Fry and Parker, 1979), but a 0.8‰ variation was noted with age (Yoneyama *et al.*, 1983). Intraorganism variation ranges averaged about 3‰ for both terrestrial and marine mammals.

Seasonal differences may result from changes in the types of food available at different times of the year or from variations in environmental conditions. Lowdon and Dyck (1974) have shown that the $\delta^{13}C$ values of maple leaves and grass species collected at a single location can vary by more than 5‰ during the growing season.

1.9.2 Influence of diet on the carbon isotope composition of animal fats

The influence of diet on the distribution of carbon isotopes in animals has been investigated by analysing animals grown in the laboratory on diets of constant carbon isotopic composition (DeNiro and Epstein, 1978). The study established that the isotopic composition of the whole body of the animal reflects the isotopic composition of its diet but that the animal is on average enriched in $\delta^{13}C$ by about 1‰ relative to its diet. It was found that the relationship between the $^{13}C/^{12}C$ ratio of a tissue and the $^{13}C/^{12}C$ ratio of the diet depends both on the type of tissue and on the nature of the diet, with differences between the $\delta^{13}C$ values of a biochemical fraction in an animal and in its diet as large as 3‰. This study also showed that isotope fractionation is consistent between different species and within species, indicating that dietary analyses based on isotopic measurements are viable.

A useful study has been carried out by Tieszen *et al.* (1983) into the fractionation and turnover of stable carbon isotopes in animal tissues. The results indicated that fat tissue was 3‰ more depleted in ^{13}C relative to the diet and showed the largest departure from dietary ^{13}C relative to other tissues due to discrimination against ^{13}C during lipid synthesis (DeNiro and Epstein, 1977). Fat tissue was found to have a relatively short half life of 15.6 days indicating that carbon turnover was relatively rapid compared to other tissues, requiring 208 days in total for complete turnover of carbon. DeNiro and Epstein (1978) showed that the fractionation of ^{13}C from diet to tissue is not identical on different diets, possibly because of differential assimilation between the major biochemical fractions of different diets, however, the secondary fractionation of carbon isotopes by animal tissues is believed to be relatively small.

Since forage provides a significant contribution to the diet of ruminant animals, the effect of contributions to the diet of different plant types has been studied in relation to the $\delta^{13}C$ values of depot fats. Recent studies have focused on differences in $\delta^{13}C$ values of C_3 and C_4 plants and their effect on fat composition (Minson *et al.*, 1975). The C_3 pathway is the most common pathway for photosynthesis and results in a lower $\delta^{13}C$ value in the fixed carbon than in the CO_2 source and operates in most higher plants, algae, cyanobacteria and some photosynthetic and chemosynthetic bacteria. A second pathway used by a small proportion

of plants involves the C_4 pathway which is a relatively recent evolutionary development, particularly encouraged by hot climates. The differences resulting from carbon isotope fractionation during photosynthesis due to differences in the enzymes that catalyse CO_2 fixation (Bender, 1971; Smith and Epstein, 1971; Bender *et al.*, 1973) are responsible for most of the isotopic variation in natural terrestrial environment (Farquhar *et al.*, 1989). Most terrestrial plant species in temperate countries are C_3 (Osmond *et al.*, 1982), however both C_3 and C_4 species are represented in salt marshes, with $\delta^{13}C$ values near -27‰ for C_3 species and -13‰ for C_4 species (Fry and Sherr, 1984). Carbon isotope fractionation in plants is reviewed by O'Leary (1981).

1.9.3 Isotope effects associated with fat synthesis

An understanding of the fractionation which occurs during lipid synthesis in the animal, including the effect of isotopic fractionation and the differences between different tissue sites and the major biochemical fractions, is of fundamental significance to this thesis. Factors controlling the stable isotope compositions of naturally synthesised organic materials have been widely discussed (Hayes, 1993; DeNiro and Epstein, 1977). In animals, isotopic distribution is a function of the relative contribution of *de novo* synthesis and assimilation of precursors from the diet. In addition, studies have suggested that changes in the $\delta^{13}C$ value result from the numerous metabolic pathways which enable the conversion of carbon from one biochemical fraction to another (Mahler and Cordes, 1971). Isotope effects during the pyruvate dehydrogenase reaction to produce acetyl CoA for lipid biosynthesis account for the ^{13}C depletion of the lipid fraction observed in organisms as they exist in nature. Oxidation of pyruvate to acetyl CoA by the pyruvate dehydrogenase complex results in a decrease in ^{13}C content of the acetyl group relative to the pyruvate due to kinetic isotope effects (DeNiro and Epstein, 1977). The ^{13}C depletion is concentrated primarily in the carbonyl carbon atom of acetyl CoA, with the methyl group retaining the $\delta^{13}C$ value of the glucose. Incorporation of the ^{13}C-depleted acetyl groups into lipid components results in the ^{13}C depletion of the lipid fraction. Species-specific differences in the magnitude of the ^{13}C depletion could arise from different kinetic isotope effects during the specific pyruvate dehydrogenase reactions and from different flow rates of pyruvate to its other metabolic reactions (DeNiro and Epstein, 1977). The sources of acetyl CoA for different animals have been discussed in Section 1.6.2, however in general, both glucose

and acetate are utilised as fatty acid precursors in monogastric animals, while acetate and lesser amounts of β-hydroxybutyrate are the two principal fatty acid precursors in the ruminant mammary gland (Swenson and Reece, 1993). Where the sole carbon source is acetate then the steps causing the isotope fractionation will be eliminated and the $\delta^{13}C$ values of the synthesised lipids will reflect those of the carbon source.

1.9.4 $\delta^{13}C$ values of atmospheric CO_2

Various factors are believed to affect changes in the $\delta^{13}C$ value of atmospheric CO_2 which ultimately determines the $\delta^{13}C$ values of the tissues of living organisms. In addition to the major changes since the Industrial Revolution through the increased burning of fossil fuels (Farmer and Baxter, 1974; Mook *et al.*, 1983), variations occur seasonally in response to patterns of photosynthesis and soil respiration in the northern hemisphere (Mook *et al.*, 1983; Keeling *et al.*, 1996; Friedli *et al.*, 1986) and spatially, with differences in the $\delta^{13}C$ value of atmospheric CO_2 from urban (-7.8‰ to -12‰) and remote (-7.8‰) areas (e.g. Levin *et al.*, 1987) and oceanic reservoir effects (Mook *et al.*, 1983). Data from Keeling *et al.* (1989) show pronounced seasonal variations of biogenic origin in the $\delta^{13}C$ values of atmospheric CO_2 with values heavier in summer, lighter in winter, with an amplitude of approximately 1‰ in high northern latitudes, decreasing towards the equator. In autumn, atmospheric $\delta^{13}C$ is maximal due to photosynthetic discrimination against ^{13}C by plants during the recent growing season. By contrast, $\delta^{13}C$ of atmospheric CO_2 is lowest in spring due to rapid soil respiration, which releases CO_2 derived from organic matter more depleted in ^{13}C than the atmosphere.

The fossil fuel effect is probably the most significant factor which influences both regional and seasonal variations in concentrations and $\delta^{13}C$ values of atmospheric CO_2. Thus, the magnitude of these variations would have been significantly less in the years preceding the Industrial Revolution. Anthropogenic CO_2 is derived from the destruction of biomass, including combustion of fossil fuels, deforestation and enhanced soil respiration from increased agriculture. All of the fossil fuels have low $\delta^{13}C$ values and their combustion produces CO_2 that is approximately -26‰, substantially lower in ^{13}C than the atmosphere (Tans, 1981). Vegetation removed during deforestation is also low in ^{13}C, so that when it decomposes or burns, ^{13}C-depleted CO_2 is released into the atmosphere. Thus, the net result

of fossil fuel combustion and deforestation is dilution of the ^{13}C content of the atmosphere. The oceanic Σ DIC pool controls the $^{12}C/^{13}C$ ratio of atmospheric CO_2 and marine carbonates.

Measurements of $\delta^{13}C$ values of individual tree rings from oak (*Quercus robur*) and larch (*Larix decidua*) have shown that a rapid decline occurred in $\delta^{13}C$ during the early 1900's with an average decline in $\delta^{13}C$ of atmospheric CO_2 amounting to 1.7‰ between 1900 and 1964 (Farmer and Baxter, 1974). Freyer (1986) calculated an average trend from all available tree ring data to obtain a change of -1.6‰ between pre-industrial times and 1980. Friedli *et al.* (1986) investigated changes in the $^{13}C/^{12}C$ ratio of atmospheric CO_2 over the past two centuries by measurements of $\delta^{13}C$ values of CO_2 separated from air trapped in bubbles in ice samples from an ice core from Antarctica. Their measurements indicate that the $\delta^{13}C$ value of atmospheric CO_2 has decreased by approximately 1.14‰ from the end of the 18th C to 1980 (the overall estimated uncertainty of this figure is ± 0.15‰) and is continuing to decline. $\delta^{13}C$ values were obtained for only three samples from before 1800, however, the trend seems to indicate more consistency in the values of CO_2 prior to 1800 which is supported by $\delta^{13}C$ values recorded for ice core samples for the South Pole (dating from 1220-1560; Friedli *et al.*, 1984). Analyses of South Pole samples have shown that CO_2 concentrations remained steady between 1200-1800 with secular fluctuations not exceeding 10 ppm.

1.10 Previous work to distinguish between animal fats

Previously, the identification of species origin has mainly been confined to the field of forensic science, where immunological (Manunza and Pappalardo, 1966), electropholic (Pflug, 1988) and DNA (Guglich *et al.*, 1994) analyses have proven invaluable techniques. However, chemical analyses have also been applied to the determination of animal fat origin based upon free fatty acid distributions (Matter *et al.*, 1989; Matter, 1992) and positional analysis of fatty acid moieties in triacylglycerols (Kagawa *et al.*, 1996). Detailed accounts of lipid distributions in different animal fats are widespread in the literature and excellent reviews have been compiled by Christie (1978) and Body (1988).

Little detailed work has been undertaken previously to classify ancient animal fats where the original lipid signature has been altered as a result of degradation, the exception being work by Morgan *et al.* (1973; 1983; 1984), Thornton *et al.* (1970) and Rottländer and Schlichtherle (1979), involving the analyses of fatty acid distributions in fatty materials from archaeological contexts. Work by Lawrence in 1994 in our laboratory showed that distinctions could be drawn between degraded fat extracts from medieval lamps and 'dripping' dishes on the basis of the positional isomers of the monounsaturated fatty acids. Further work (Mottram, 1995; Evershed *et al.*, 1997a) showed that the natural variation in $^{13}C/^{12}C$ ratios of *n*-alkanoic acids in animal fats enables distinctions to be made between a marine or terrestrial source, and furthermore, the identification of a ruminant or non-ruminant origin for archaeological fats. These studies proved to be a break through in the determination of animal fat origin and have since been further developed, making a significant contribution to this thesis.

An extensive study of the fatty acid composition of native food plants and animals of Western Canada has recently been undertaken by Malainey *et al.* (1999a, b). Distinctions are made between the major food groups using cluster and principal component analysis. The study was carried out in order to build a data base of possible foodstuffs in order that comparisons may be made with the absorbed residues from vessels used by the peoples of Western Canada during the Late Precontact Period. Although this work provides a painstakingly detailed account of the similarities and differences between fatty acid profiles of different species, and enables distinctions to be drawn between large mammal fat, large herbivore meat, fish, and plant roots, amongst other food groups, the distinctions are based largely upon abundances of mono- and polyunsaturated free fatty acids. It is commendable that the same workers also consider the effect of thermal and oxidative degradation on these fatty acid distributions (Malainey *et al.*, 1999c), however, the complexities of chemical, biological and physical decay processes upon the ratios of the different fatty acids have not been given ample consideration, and it is wholly unrealistic to believe that identifications of origin can be made based purely upon the relative proportion of saturated and unsaturated fatty acids present in an archaeological residue in which the extent of decay is unknown.

1.11 Aims of this thesis and experimental design

Our ability to obtain detailed information at the molecular level from lipid residues associated with archaeological artefacts has dramatically increased the potential for deriving invaluable information on Man's use of natural commodities in past societies. Since a high proportion of the archaeological potsherds analysed in our laboratory have contained animal fats, a major question which arose was: "What further chemical information could be obtained from these fats which would enable the identification of species origin?". Direct information on the types of animals exploited for their fat and meat would prove invaluable in the study of site economy and way of life in antiquity, particularly at sites where other environmental and artefactual evidence is lacking. A new approach involving detailed compositional analysis of remnant fats was clearly required to retrieve new information which could enable an insight into animal exploitation, dietary preferences and vessel use in prehistoric Britain.

The new approach has involved the use of a combination of chemical criteria to characterise the origins of animal products recovered from prehistoric archaeological sites. The initial stages of this study involved the screening of several carefully chosen assemblages of pottery, from the Neolithic to Late Saxon/early medieval periods, in order to establish the presence (or absence) of organic residues. Specific chemical criteria were applied to the archaeological fats and comparisons made with modern reference animal fats. The same chemical criteria were applied to remnant fats from ethnographic vessels and assemblages from archaeological sites with a particularly strong bias in the faunal record in order that identifications based on comparisons with modern reference fats could be supported by comparisons with degraded fats of known origin. The ultimate goal of this work was to apply the chemical criteria to remnant fats in assemblages from earlier, prehistoric, sites from where little or no faunal evidence had been found, any chemical evidence obtained thus comprising the only information available on the types of animals exploited for their meat, fat and secondary products (e.g. milk or hide) at these sites. Interpretations of vessel function will be used to identify patterns of utilisation of the major domesticated animals at different sites and correlations will be made with the results of

faunal studies (where available). Consideration will also be given to the correlation of vessel form, where known, with vessel contents.

In this study, chemical analyses based on solvent soluble acyl lipid components have comprised the study of:

(i) Overall lipid distributions;

(ii) Relative abundances of *n*-alkanoic and branched-chain fatty acids;

(iii) Relative abundances of positional isomers of the monounsaturated C_{18} fatty acid;

(iv) Distributions of intact triacylglycerols;

(v) $\delta^{13}C$ values of individual $C_{16:0}$ and $C_{18:0}$ fatty acids.

Throughout this work consideration has been given to various questions relating to the robustness of the parameters used and also the reliability of the results obtained. Effects of diagenesis have been investigated, including possible changes in the compositions of lipid components due to decay over archaeological time (investigated through laboratory decay experiments using fats and pure components) and the possible contribution of micro-organisms and bacterial reworking and decay to the original lipid profile.

CHAPTER 2

Modern Reference Materials and Archaeological Sites and Samples

2.1 Reference materials

2.1.1 The selection of modern animal fats as reference materials

The majority of farmed animals available today are unsuitable for comparison with archaeological fats due to the changes which have occurred in the composition of animal fats over the last several hundred years. Reasons for this include: (i) selective breeding, which has resulted in changes in the composition of the fat and milk of the larger domestic animals (Johansson and Claesson, 1957); (ii) the widespread use of intensive farming methods necessary to maximise milk yields, which has included the use of nutrient-rich concentrates during the winter when temperatures are low (Johansson and Claesson, 1957, and references therein); (iii) fossil fuel burning since the Industrial Revolution (Section 1.9.4), and other factors resulting in changes in the isotopic composition of the atmospheric CO_2 which have been reflected in the enrichment of ^{12}C in the tissues of modern animal fats compared to their ancient counterparts, and (iv) C_4 plants (sugar cane) introduced into Europe in the 1500's, which have been incorporated into the diets of farm animals significantly altering the stable carbon isotope composition of the animal tissues.

In view of the above, reference animal fats for this study have been carefully selected from a number of sources, including:

(i) Fresh fats from modern animals raised on a known diet;
(ii) Remnant fats extracted from well-documented ethnographic vessels;
(iii) Remnant fats from archaeological pottery assemblages at sites where a preponderance of one species of animal is believed to have been farmed;
(iv) Remnant horse fats obtained from a prehistoric permafrost burial.

2.1.2 Modern reference samples

Tables 2.1 to 2.10 below detail the modern reference fats sampled. In addition to fats from animals most likely to have been the major species exploited in antiquity in Britain, examples of salt water fish and virgin olive oil have been analysed for comparison. The procedure for analysis of reference fats and oils is given in Section 9.1.2. The reference animals were raised on diets isotopically representative of the archaeological periods studied, with the exception of the cows raised for commercial milk production (referred to in Section 6.2) which were fed concentrate supplements.

Table 2.1 Cattle depot fats.

Sample	Type	Breed	Part	Age	Diet
Cow 1 (C1BB)	Sub.[1]	Hereford/ Fresian beef steer	Brisket	24 mths	UP[2]
Cow 1 (C1BR)	Sub.	Her./Fres. beef steer	Rump	24 mths	UP
Cow 1 (C1BK)	Int.[3]	Her./Fres. beef steer	Kidney	24 mths	UP
Cow 2 (C2BB)	Sub.	Highland beef steer	Brisket	26 mths	UP

[1] Subcutaneous adipose
[2] Unimproved pasture
[3] Internal fat

Table 2.2 Cattle milk fats.

Sample	Type	Breed	Part	Age	Diet
Sarah 1	Milk	Dexter	Pre-birth	-	UP
Sarah 2	Colostrum	Dexter	Post calving	-	UP
Mallard 1	Milk	Dexter	Pre-birth	-	UP
Mallard 2	Milk	Dexter	2 weeks post calving	-	UP
Twinkle 1	Milk	Charalais/ Jersey	1 day post calving	9 yrs	UP
Twinkle 2	Milk	Charalais/ Jersey	3 weeks post calving	9 yrs	UP
Tulip 1	Colostrum	Dexter heifer	Post calving	17 mths	UP
Tulip 2	Milk	Dexter heifer	3 days post birth	17 mths	UP

Table 2.3 Sheep depot fats.

Sample	Type	Breed	Part	Age	Diet
Mutton 1	Adipose	Hebridean	-	4-5 years	UP
Mutton 2	Adipose	Hebridean	Leg fat	-	UP
Mutton 3	Adipose	Hebridean	Shoulder fat	-	UP
Heb lamb 1	Adipose	Heb. lamb	Breast fat	10 mths	UP
Ram lamb 1 (10 months)	Adipose	-	-	-	UP
Ram lamb 2 (10 months)	Adipose	-	-	-	UP
Ewe 1	Adipose	-	-	-	Grass
L1B91[4]	Adipose	-	Back fat	-	
L1K91[4]	Sub.	-	Kidney fat	-	
L2B91[4]	Adipose	-	Back fat	-	
L2K91[4]	Adipose	-	Kidney fat	-	
L3B91[4]	Adipose	-	Back fat	-	
L3K91[4]	Adipose	-	Kidney fat	-	

[4] Fats collected from abattoir; dietary history is not known.

Table 2.4 Sheep milk fats.

Sample	Type	Breed	Stage	Age	Diet
Heb 1	Milk	Hebridean ewe	-	-	UP
Heb 2	Milk	Heb./Soay ewe	Late lactation	2-3 yrs	UP

Table 2.5 Deer depot fats.

Sample	Type	Breed	Part	Age	Diet
Deer 1 (D1BB)	Sub.	Red deer female	Breast	-	UP
Deer 1 (D1BR)	Sub.	Red deer female	Rump	-	UP
Deer 1 (D1BK)	Int.	Red deer female	Kidney	-	UP
Deer 2 (D2BB)	Sub.	Red deer female	Breast	22 mths	UP
Deer 2 (D2BR)	Sub.	Red deer female	Rump	22 mths	UP
Deer 2 (D2BK)	Int.	Red deer female	Kidney	22 mths	UP
Deer 3 (D3BB)	Sub.	Red deer female	Breast	22 mths	UP
Deer 3 (D3BR)	Sub.	Red deer female	Rump	22 mths	UP
Deer 3 (D3BK)	Int.	Red deer female	Kidney	22 mths	UP

Table 2.6 Pig depot fats.

Sample	Type	Breed	Part	Age	Diet
Pig 1-8	Sub.	Hampen/large white boar	Tail fat	16 wks	Barley meal
C_3-fed pig	Sub.	Hampen/large white boar	Tail fat	16 wks	Barley meal

Table 2.7 Goose depot fat.

Sample	Type	Breed	Part	Age	Diet
Goose 1-4	Adipose	-	-	-	Pellets and grass

Table 2.8 Chicken depot fat.

Sample	Type	Breed	Part	Age	Diet
Chicken 1-9	Depot fat	Male broilers	Abdominal	3 weeks	Pellets

Table 2.9 Horse depot fats.

Sample	Type	Breed	Part	Age	Diet
H1PP	Int.	Thoroughbred female	Peritoneal	19 yrs	Mixed
H1PL	Sub.	Thoroughbred female	Leg	19 yrs	Grass
H3PP	Int.	Thoroughbred female	Peritoneal	17 yrs	Grass
H3PL	Sub.	Thoroughbred female	Leg	17 yrs	Grass
H4PP	Int.	Gelding cross	Peritoneal	15 yrs	Grass
H4PL	Sub.	Gelding cross	Leg	15 yrs	Grass
H5PP	Int.	Thoroughbred cross female	Peritoneal	14-15 yrs	Grass
H5PL	Sub.	Thoroughbred cross female	Leg	14-15 yrs	Grass

Table 2.10 Fish and plant oils.

Sample	Type	Breed	Part
FC1	Salt water fish	Cod	Flesh
FH1	Salt water fish	Haddock	Flesh
FP1	Salt water fish	Plaice	Flesh
OOIL 1	Olive oil	Pure virgin	-

2.2 The pottery assemblages

2.2.1 Sites with well documented faunal assemblages

Archaeological sites were specifically selected in order to enable a systematic approach to identifying the origins of animal fats. The first assemblages investigated came from relatively recent sites with well-preserved, well-documented faunal evidence, namely the Late Saxon/early medieval settlement of West Cotton, Northamptonshire and the Iron Age/Romano-British settlement of Stanwick, Northamptonshire.

2.2.1.1 West Cotton (Late Saxon/early medieval)

West Cotton comprises a Late Saxon and medieval hamlet with underlying Bronze Age barrows and a Neolithic long enclosure, all excavated as part of the Raunds Area project alongside Stanwick. The three main phases of settlement included a Saxon occupation dating from *ca* 950 to 1100 AD, a medieval settlement and manor between 1100 and 1250 AD and during the final phase, between 1250 and 1450 AD, a later medieval manor and

hamlet. Overall, West Cotton would have comprised a less grand settlement than the Romano-British settlement at Stanwick.

The faunal remains at West Cotton have been reported by Albarella and Davis (1994) and percentages of the main species are given in Table 2.11. All the caprine bones at the site had belonged to sheep, so no goat were present. Sheep are thought to have been the most common food species at West Cotton (Albarella and Davis, 1994). Butchery marks in cattle were common and can be related to the severing of tendons. The cut and chop marks on equine bones were surprisingly frequent at West Cotton, so horse meat may have been exploited, e.g. for feeding dogs. The faunal report suggests no bias in the representation of different parts of the skeleton of species as a result of human activity, and thus it has been suggested that all animals were reared, slaughtered and butchered on site.

Table 2.11 Minimum numbers of individuals (n) of the four main species in the main levels at West Cotton.

SPECIES	**PERIOD**					
	Early medieval		**Middle medieval**		**Late medieval**	
	n	%	n	%	n	%
Cattle	37	**26**	20	**20**	7	**13**
Sheep	69	**48**	63	**62**	37	**66**
Pig	31	**22**	12	**12**	7	**13**
Equid	7	**5**	7	**7**	5	**9**

In the medieval period, sheep represent the most common taxon and their frequency even increased in the mid-late medieval period (Albarella and Davis, 1994). It is believed that sheep were probably kept mainly for the production of wool but that milk and meat were also used. The increased number of adult animals in the later period can be related to the increasing importance of wool production. Goats appear to be completely absent from the medieval period. Cattle were probably used for traction as well as meat and dairy products but decreased in number in the course of the middle ages, probably as a consequence of the increasing importance of sheep and perhaps also because some of the work oxen were replaced by horses. It has been tentatively suggested that a higher number of juvenile cattle were killed in the late medieval period; this may indicate an increase in beef production and decreased use of cattle as work beasts. Pigs were clearly exploited for meat and lard, as

indicated by the high number of immature animals, however, numbers also decreased in the mid-late medieval period, perhaps also as a consequence of the increased number of sheep or the countrywide contraction of woodland. Overall, the faunal evidence has suggested that the major changes in the pattern of exploitation of animal resources which occurred between the two main medieval periods were the increased importance of wool and horse power. However, these were probably country wide trends, as documented in both historical and archaeological sources, which may have been influenced by the transformation over these periods of the site from a manor to a hamlet.

2.2.1.2 Stanwick (Iron Age/Romano-British)

Stanwick is a multi-period site on the River Nene, Northamptonshire, the excavation of which was funded and managed jointly by English Heritage and the Northamptonshire County Council Archaeology Unit, with the aim of investigating settlement and land use at the site. Essentially, the site consisted of several major phases beginning with late Iron Age occupation evidenced by several huts and a palimpset of pits and drainage gullies. Plant remains suggest a strong pastoral element in the Iron Age environment with pollen grain analyses suggesting a very open site dominated by grasses and herbs. The apparently cohesive community of the Iron Age settlement appears to have developed into a series of increasingly independent, self supporting farmsteads by the Romano-British period. The major phase of Roman activity began in the 2nd Century AD, from which period an extensive Roman settlement and villa have been unearthed with evidence that Stanwick was a relatively high status site. A large number of coins (ca. 5000), abundant pottery vessels, sculptures, broaches and other small finds were recovered from the site. Corn-dryers and fragments of rotary querns in the locality indicate the importance of crop processing.

The abundant faunal remains (Table 2.12) of sheep, goats, pigs and cattle as well as horses and dogs confirm the farming of livestock in the Romano-British period. Hunting of wild animals and fishing were evidenced by Roe and Red deer remains and the presence of spear tips, and the discovery of wool combs, sheers and other related implements indicate the processing of fleeces and the production of textiles. A full report discussing the significance of the faunal evidence from Stanwick is in preparation by English Heritage.

Table 2.12 Faunal remains at Stanwick (% occurrence).

DATE	SPECIES				
	Sheep/goat	**Cattle**	**Pig**	**Horse**	**Other**
Iron Age	47	38	6	8	1
3rd-4th C AD	37	38	11	7	7

2.2.2 Sites with an unusually strong bias in the faunal record

Assemblages from two archaeological sites were selected specifically because a particularly strong bias exists in the type of faunal remains recovered, namely Wicken Bonhunt, Essex and Fuller's Hill near Great Yarmouth, Norfolk. It has been suggested that Wicken Bonhunt functioned as a breeding and distribution centre for pigs in the Middle Saxon period. Therefore, sherds from relevant contexts from this site , i.e. those associated with abundant pig bone, were selected for analysis in order that residues may be recovered which represent remnant porcine fats. In addition, samples were collected from Fuller's Hill where there is clear implication that pottery would have been associated with fish preparation and consumption (Rogerson, 1976).

2.2.2.1 Fuller's Hill (Late Saxon/early medieval)

The proto-urban to urban settlement at Fuller's Hill, Great Yarmouth, excavated between 1971 and 1973, yielded many hundreds of potsherds of domestic vessels in association with large numbers of fish bones (Rogerson, 1976). The pottery vessels represented 'cooking' pots, bowls (shallow and deep-sided), ginger jars, lamps, storage jars and pitchers. Sherds were mostly well-stratified, although fragmentary, particularly in the earlier phases and have been dated to between the late 11th and early 13th C. Seventy percent of the coarse wares were tempered with natural alluvial detritus, the remainder being tempered with flint, sand and shell. The finer wares were imported to the site with the Late Saxon/early medieval wares showing the trade connection between Great Yarmouth and the continent in the late 11th and 12th Centuries. Fish remains were collected by sieving and 19 species of mainly salt water fish were identified, including Herring, Cod, Whiting and Mackerel as the dominant species and lesser quantities of Plaice, Haddock and Conger eel. In addition to the fish bone, large quantities of plant remains were recovered, comprising cereals, mainly oats, barley and bread wheat and also peas and beans in small quantities. The animal bone from the site was extremely fragmentary, thought to be as a

result of butchery. However, in general, sheep were the predominant species with varying proportions of pig and ox. Substantial quantities of bird bone were also present. The vessels from Fuller's Hill provide excellent material on which to test the ability of GC-C-IRMS to separate fats of marine and terrestrial origin since fishing is believed to have provided the basis of the economy at this site, its importance evidenced by the large number of fish bones and hooks recovered.

2.2.2.2 Wickham Bonhunt (Romano-British/Middle Saxon)

Considerable evidence has been found for prehistoric, Romano-British and Middle Saxon settlements at Wicken Bonhunt, with substantial activity during the latter period which is believed to have comprised at least two major phases (Wade, 1980). Ditches from the Middle Saxon settlement have provided artefactual evidence for a rural economy and evidence was found for at least 28 structures, with the layout of the site indicating a highly organised settlement. Soil samples from Roman and Middle Saxon contexts were passed through a flotation tank to reveal a similar range of cereals from each period, comprising mainly bread wheat with some oats, barley, peas and beans. The faunal and pottery assemblages recovered from this site were well preserved, with pottery the most common find. The pottery assemblage from the Middle Saxon period comprised mainly Ipswich ware (70%), handmade grass-tempered and shell-tempered ware (20%) and imported continental wares (10%).

The faunal assemblage from the Middle Saxon period comprised 102,751 animal bones and fragments, with the majority recovered from a boundary ditch. Surprisingly, domesticated pigs make up nearly 70% of the large domestic mammal remains which is in contrast to other comparable sites of this period where cattle or sheep bone dominate the faunal assemblages. In fact, Wicken Bonhunt is the only Anglo-Saxon site in East Anglia that has produced a predominance of pig remains, with bones comprising a high proportion of pig cranial elements. Age estimates have shown relatively few neonates or young juvenile animals, with the most common age of death being approximately 3 years of age (Grant, 1982).

Studies of mandibular remains have indicated that Wicken Bonhunt served as a pig-breeding site with many of the younger animals being exported to other sites, perhaps forming part of a broader network of trade and exchange in animal products (Crabtree, 1995). It is not clear, however, whether the meat products were being used locally for feasting, warfare, etc., or whether the products were being exported to larger contemporary population centres such as Ipswich or London. Cattle bones were also found at Wicken Bonhunt, although not in such high numbers and were mainly from mature and elderly animals, indicating that cattle were kept for purposes other than meat and milk, possibly as plough oxen. Sheep make up less than 20% of the assemblage, with horse bone comprising less than 1% but showing butchery marks suggesting that they may have formed part of the Middle Saxon diet. A large group of bird bones were also identified, including 295 fowl and 228 geese.

2.2.2.3 Botai, Kazakhstan

Botai is an early Neolithic settlement, dated around 3500 BC (Levine and Kislenko, 1997) and located in Kokchetau Oblast in the forest-steppe region of northern Kazakhstan. Botai comprised around 300 semi-subterranean, polygonal 'dwellings', packed closely together in a pattern reminiscent of a honeycomb, and oriented in parallel rows on either side of 'streets', 4 to 8 meters wide (Zaibert, 1993). Over 140 of these structures have been excavated to date, ranging in area from 30 to 70 square metres. It has been estimated that over the course of 15 years of excavations at Botai, more than 300,000 artefacts and 10 tonnes of bones have been recovered. Astonishingly, 99.9% of the faunal remains are derived from horses. There are differing opinions as to whether the horses were wild (Ermlova, 1993) or whether they were domesticated for riding and meat production (Zaibert, 1993). On the basis of a detailed analysis of population structure, Levine (in press) concluded that the vast majority, if not all, of the horses studies were from a hunted population and were probably killed in herd drives. Limb bones and sections of vertebral columns were found articulated, indicating that butchery was not very intensive and considerable waste had been tolerated. During the excavations, around 12,300 ceramic sherds were recovered from Botai, typically highly decorated and made of local clay.

2.2.3 Prehistoric archaeological sites

Following the analysis of samples from the Romano-British, Saxon and medieval periods, the focus of the research shifted to earlier, prehistoric sites, where prolonged burial, intensive ploughing of soils and the effects of strongly alkaline or acidic burial conditions frequently result in very poor preservation of faunal and environmental remains. The rarity of representative organic remains at prehistoric sites (with the exception perhaps of permanently waterlogged sites) somewhat limits the use of traditional investigative techniques in determining the types of animals farmed and species of plant materials available.

The same chemical criteria applied to the assemblages already described have been used to draw distinctions between fats of different origins from prehistoric sherds. The aims were: (i) to provide evidence relating to commodity use and animal exploitation at early sites, and (ii) to investigate the degree of preservation of lipid residues which have been decaying for several millennia. Five assemblages have been sampled, including two assemblages from Yarnton, Oxfordshire, where bone preservation was variable, and two assemblages from Eton, Buckinghamshire. Environmental and faunal remains from the early site at Eton were very poorly preserved in comparison to finds recovered from the later prehistoric site. The fifth assemblage is from Upper Ninepence in the Welsh borderlands which yielded a small assemblage of pottery from two Neolithic dates, however, the bone preservation at this latter site was extremely poor and thus no information is available concerning the animal species exploited.

2.2.3.1 Yarnton Cresswell field (early-middle Iron Age)

The Iron Age features at Yarnton Cresswell field are believed to be predominantly related to domestic activity but, due to the fragmentary nature of the pottery assemblage, in most cases the exact form of the vessel from which the sherds derive is unknown. However, two vessel types from context 8126 have been identified, with one sherd deriving from a bowl form (sample no. 126; Plate I) which is very unusual for this region both in form and decoration (but not in fabric). It is originally believed to have come from Wessex and not the Thames Valley where it was found. The unspecified base and body sherds from the second vessel are thought to be almost certainly derived from one of the common forms,

i.e. barrel-shaped jar, slack shouldered jar or tripartite jar (Paul M. Booth, pers. comm.; Plate II). These vessel forms are potentially multi-functional domestic wares, however, the tripartite jars are often made of slightly finer fabrics and better finished than other vessels and conceivably represent 'table ware' from the early Iron Age. The vessels are considered to be significant since they were recovered from the fill of one of the earliest Iron Age features on the site which contained an unusually large group of pottery dating to the 7th C BC.

Cresswell field lies on calcareous gravels which have afforded good preservation to bone, and thus a large faunal assemblage was recovered in excellent condition, which comprised mainly cattle (64%) but also sheep and goat (27%), pig (7%) and horse (2%), but very few wild animals. Butchery marks were visible on some bone. Charred plant remains included wheat (hulled and free-threshing) and barley. Grain and processing waste were found in the early Iron Age levels, comprising mostly spelt wheat with some barley. More processing waste was identified in the middle Iron Age with evidence for increasing agricultural intensity and harvesting of lowland straw. It is anticipated that the excellent preservation afforded to the animal bone and vegetable material will also be reflected in the abundance and quality of organic matter retained by the potsherds; it would be expected that fats derived from the processing of animal products should be well-preserved.

2.2.3.2 Yarnton flood plain (Neolithic-Bronze Age)

Excavations of the Neolithic-Bronze Age settlement on the flood plain recovered evidence of domestic, funerary and ceremonial activity dating from the early Neolithic until the end of the Bronze Age, with features including a long mortuary enclosure, a beaker burial and several Bronze Age circular buildings. This activity was situated on low gravel islands and in adjacent palaeochannels subsequently buried beneath alluvium and frequently underwater since the Late Bronze Age. Vessel contents and function may vary according to their context and their date of deposition (there is evidence of domestic activity from at least the middle Neolithic). The faunal assemblage from the flood plain was poorly preserved compared with the faunal remains from Cresswell field, but included the major domestic species, principally cattle but also sheep with small numbers of pig, horse and deer. Charred plant remains included hazelnuts, apples and other wild plant foods but

Plate I

Plate II

SCALE (cm)

comparatively few cereal grains. It will be interesting to compare the results of residue analysis of vessels from the assemblages from Yarnton Flood Plain and Yarnton Cresswell field, and to note any differences which may relate to the transition of settlement from the Late Bronze Age to the early Iron Age. The latter is thought to be associated with greater permanence of settlement, a shift to arable agriculture and more intensive exploitation of the land.

2.2.3.3 Eton Lake End Road (late Neolithic-Early Bronze Age)

Prehistoric activity has been identified at the Lake End Road site at Dorney, including a Middle Bronze Age cremation. Three hundred and forty nine sherds (minimum of three vessels) of Middle Bronze Age pottery were recovered and can be assigned to the Deverel-Rimbury tradition. The vessels include a single Bucket Urn with unusual decoration. Nine late Neolithic pits produced exceptionally large assemblages of Peterborough ware pottery and worked flint, including part of a polished flint axe head. Environmental samples from the pits produced significant quantities of hazelnut shells with only occasional cereal or cultivated legume remains. A total of 1811 sherds were recovered from Neolithic pit deposits and surface spreads, including an outstanding group of later Neolithic Peterborough ware (50-100 vessels) associated with lithic material. Burnt residues were noted on a number of the Peterborough ware sherds. Pit groups contain mostly Mortlake vessels, with one pit of Fengate ware. In addition, an excavated hollow contained an assemblage of earlier Neolithic plain bowl. Later Neolithic pits produced hazelnut shell fragments (*Corylus avellana*) indicating a collected woodland component to the diet, cereal remains of free-threshing and hulled *Triticum* sp. (wheat), less well preserved *Hordeum* sp. (barley) and a single cultivated legume (context 1224). Charcoal samples included the remains of *Pomoideae* sp. (hawthorn, apple etc.), *Quercus* sp. (oak) and *Corylus/Alnus* sp. (hazel/alder).

2.2.3.4 Eton Rowing Lake (early Neolithic)

The Eton Rowing lake site at Dorney, Buckinghamshire, is a site covering approximately 150 ha. comprising a gravel terrace occupied during the Mesolithic, Neolithic and Early Bronze Age periods. Excavations carried out in 1996, yielded a linear hollow marking the course of a glacial channel, top-filled with a clayey silt. The exposed surface of the hollow

was found to be extremely artefact-rich, yielding 2260 struck flints, 2000 sherds of Neolithic pottery and 320 fragments of animal bone. The pottery was mainly round-bottomed plain bowl, but included some Ebbsfleet ware, suggesting a middle Neolithic date. Some vessels had clearly been deposited more than half complete and in general the pottery was very well-preserved. In contrast, animal bone was poorly preserved and no seeds were recovered by flotation. Surprisingly, no evidence was found for structures or hearths, thus this deposit appears to be a midden of considerable proportions. Table 2.13 lists the faunal remains identified from the midden.

Table 2.13 Neolithic midden faunal remains from Eton Rowing Lake

Species	No. of bone fragments	Relative abundance (%)
Cattle	240	61.5
Cattle (probably)	4	1.0
Sheep	1	0.3
Sheep (probably)	1	0.3
Goat (probably)	1	0.3
Sheep /goat	65	16.7
Pig	35	9.0
Pig?	6	1.5
Horse	1	0.3
Red deer	18	4.6
Red deer?	5	1.3
Roe deer	3	0.8
Deer	4	1.0
Deer?	1	0.3
Fox	1	0.3
Badger	4	1.0

2.2.3.5 Upper Ninepence (early-late Neolithic)

Upper Ninepence was a major excavation carried out by the Clwyd Powys Archaeological Trust (CPAT) as part of the Walton Basin project, comprising a Bronze Age barrow which covered a number of pits and postholes representing a Neolithic settlement. The excavation of the Neolithic pits revealed deposits of flint, ceramics and charred plant material. These were thought to be derived from a domestic context due to the presence of waste flint debris and well-used scrapers as the predominant tool type. Microwear analysis of the lithic artefacts suggests that, amongst other things, leather and bone working were carried out at

the settlement. In the Grooved ware phase, archaeological finds were more numerous, with more extensive pit digging and structural activity. Locally-made Grooved ware, flint and carbonised debris similar to the Peterborough ware phase were recovered from pits and stake-holes (Gibson, forthcoming). Due to the absence of faunal remains in any of the features, there was no direct evidence at the site to indicate animal husbandry, or indeed, to enable the identification of any animal exploitation at this site. The fundamental difference between the Peterborough and Grooved ware phases is that the two pottery types were mutually exclusive. The Peterborough ware was found associated with the earlier period (*ca* 3000 BC), whilst the Grooved ware was exclusively found in the later Neolithic contexts (*ca* 2500 BC). Radiocarbon dates indicate that the two periods are unlikely to have overlapped (A. Gibson, pers. comm.). Plates III and IV show examples of the Grooved ware sherds excavated from the site, illustrating the thick black carbonised surface residues adhering to the sherds and the grooved decoration.

2.3 Ethnographic vessels

Four ethnographic vessels (A, B, F and G) have been kindly loaned by Olga Kalentzidou from the Department of Anthropology, Indiana University, USA. A fifth vessel (C) was acquired separately by Dr Richard Evershed. It was anticipated that lipid residues from these vessels could be used as reference materials since their culinary function is well documented. Collectively, they include five vessels from Greece, including a tsoukali and two bantia forms, and one pithos vessel which originated from Turkey but was recovered as an import to Crete. The ethnographic vessels are described in Table 2.14 and illustrated in Plates V to IX.

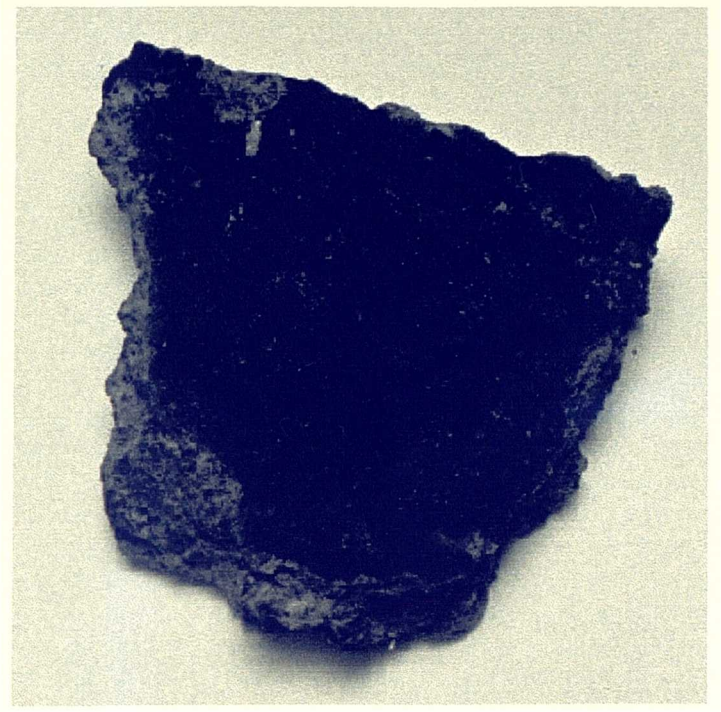


Plate III

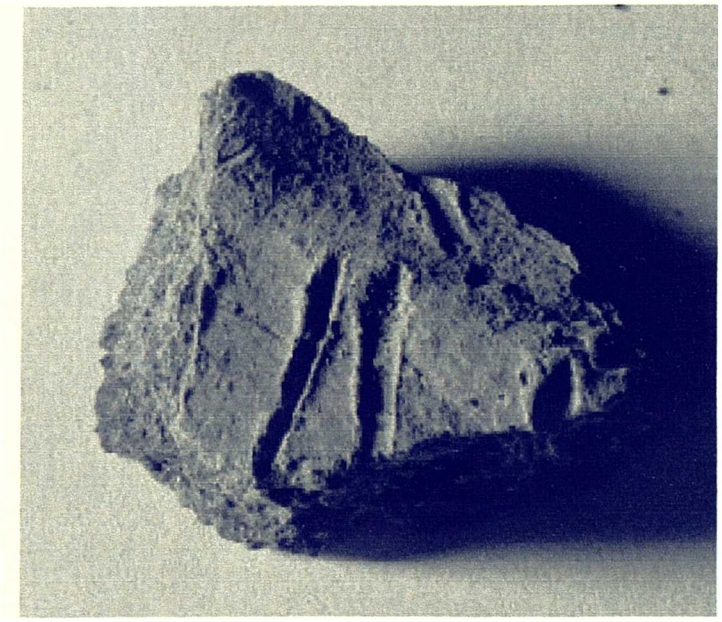


Plate IV

SCALE (cm)

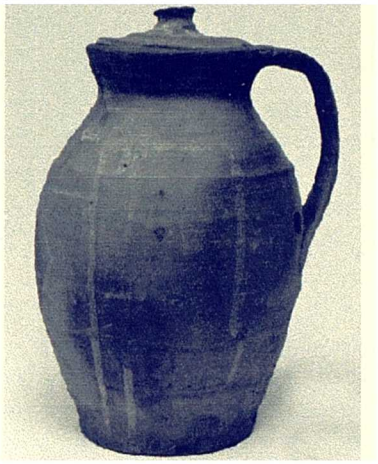


Plate V

Plate VI

Plate VII

Plate VIII

Plate IX

Table 2.14 Description of ethnographic vessels.

Vessel	Vessel description and function	Plate no.
A	Tsoukali type, made in Metaxades, Greece, 45 yrs old, used for cooking a dish called *yuvetsi*, containing pork meat, tomato sauce, oregano, salt and pepper.	V
B	Bantia type, from Metaxades, Greece, used between 1938-1970 for storing cheese, milk (with yeast and salt) and at other times used to preserve grapes with hardaki (mustard seed).	VI
C	Vessel of Turkish origin, approximately 200 yrs old, used for the storage of olive oil.	VII
F	Bantia type vessel made in Metaxades, Greece, in use between 1935-1960 for storage of *kavurmas*, a local delicacy comprising a mixture of pork fat, pork meat, salt, paprika, black pepper, oregano, onions and bahari (spice). *Kavurmas* would have been prepared at Christmas and it would last until the end of spring or early summer.	VIII
G	Vessel acquired from Milia, but probably made in Soufli, Greece, used from 1938-1960 for milk and cheese storage. The pot would have been carried to the fields occasionally where milk was consumed.	IX

2.4 Tissue remains from prehistoric Siberian horses

Samples of horse tissues and fats were provided from two of the six horses recovered from the burial of the 'Ice Princess' in the Altay Mountains. Excavated in 1993, the horses were found in an excellent state of preservation in a tomb on the high pastures of the Ukok Plateau. The tomb has provided invaluable information into the culture of the Pazyryk people of Southern Siberia, semi-nomadic horsemen who hunted and shepherded and who thrived during the 6th through to the 2nd Century BC (Polosmak, 1994). The horses were found pressed against the chamber's outer wall with patches of hair intact. Their stomachs contained a partly digested mix of grass, twigs and pine needles, identified as spring vegetation. Since horses could be used for transportation, work-force, food and warfare, the domestication of horses would have significantly influenced human ecology, social behaviour and economy (Levine, 1998). Inside the tomb, the symbolic 'last meal' consisted

of a large chunk of horse meat with a knife sticking from it and a wooden vessel thought to contain a dairy product. The Pazyryk people are believed to have consumed *koumiss*, fermented mare's milk, still drunk in central Asia.

The samples provided for analysis from the excavation were in an excellent state of preservation but with indications of adipocere formation in the subcutaneous fats. They included the following:

1. Stomach lining and meat (horse 1)
2. Skin and subcutaneous fat (horse 1)
3. Stomach contents (predominantly grass; horse 1)
4. Sacrum meat and crumbled flesh associated with the coccygeal vertebra (horse 2)

Results of the total lipid analyses by high temperature GC and GC/MS of archaeological and ethnographic vessels and horse tissues are described in the following Chapter.

CHAPTER 3
Total Lipid Analyses of Archaeological and Ethnographic Samples

3.1 Archaeological potsherds

3.1.1 Sites with well-documented faunal assemblages

3.1.1.1 West Cotton (Late Saxon/early medieval)

The initial analyses of lipids in sherds from West Cotton was carried out by Charters (1996). A total of 123 sherds from 73 reconstructed vessels were analysed initially for lipid residues, 30 of which have been selected for further analysis as part of this study on the basis that they contained appreciable abundances of animal fat residues. A detailed description of the pottery from West Cotton is given by Charters (1996), including diagrams of reconstructed vessels and information on the fabric types. Extensive work has been carried out to interpret the significance of relative abundances of lipid residues in specific regions of vessel profiles in order to investigate vessel function and methods of food preparation, i.e. boiling and roasting (Charters, 1996; Charters *et al.*, 1993b, 1995, 1997; Evershed *et al.*, 1995a). Specific vessel forms were found to correlate with lipid content, with jugs and large bowls containing no absorbed lipid, suggesting their use as carriers for aqueous liquids, although it has been suggested that the latter were of such regular volume that they may have been used for the measurement of grain (P. Blinkhorn, pers. comm.). Spouted bowls all contained remnant animal fats, and one in-turned rim bowl and a large jar contained both beeswax and animal fat residues, suggesting common functions for these vessel forms (Charters, 1996).

Many of the West Cotton vessels analysed by Charters (1996) were found to contain animal meat/fat and distributions of components characteristic of the epicuticular leaf waxes of higher plants. One commonly recognised distribution of these latter components included three long-chain alkyl compounds identified as the C_{29} *n*-alkane (nonacosane) and its oxygenated derivatives nonacosan-15-one and nonacosan-15-ol (Fig. 1.2). These components are characteristic of *Brassica oleracea* (Cabbage; Purdy and Truter, 1963; Netting and Macey, 1971) and this work illustrates one of the first identifications of organic residues from archaeological pottery to the species level (Evershed *et al.*, 1991). Additional evidence for the processing of leafy vegetables comes from the identification of the C_{31} ketone and the C_{31} *n*-alkane in several of the West Cotton vessels. These components are characteristic of the epicuticular leaf wax of modern leek (*Allium porrum*; Evershed *et al.*, 1992b, 1995b; Raven, 1995; Raven *et al.*, 1997). Documentary evidence

supports the chemical evidence for the consumption of cabbage and leek at West Cotton during this period (Henisch, 1976).

The extracts selected for use in this study are listed in Table 1, Appendix 1, p. 333. Lipid residues range in abundance from 22 μg g^{-1} to 4.8 mg g^{-1} dry weight of sherd, with a mean lipid content of 1.4 mg g^{-1}. Mono-, di- and intact triacylglycerols are present in most of the remnant animal fats, however, the majority of lipid present comprised free fatty acids, reflecting the extent to which the original intact acyl lipids have been hydrolysed. The residues are predominantly composed of degraded animal fats, although minor components characteristic of leafy vegetables were also present (Table 1, Appendix 1, p. 333).

There is extensive evidence from sooting and blackened patches that many of the vessels were heated during processing of their contents, with mid-chain ketones C_{31}, C_{33} and C_{35} (formed by heating fats in ceramic vessels at temperatures >300 °C; Evershed *et al.*, 1995b; Raven *et al.*, 1997) detected in vessels RP4, RP6 and RP89. Since the mid-chain ketones formed whilst the vessel was in use or during its failure and are relatively resistant to decay compared with other lipid components, the distribution of ketones will reflect the distribution of fatty acid components originally present in the fat, whilst the ratio of free acids is more likely to be diagenetically altered over time. Tentative identifications of residues from the West Cotton assemblage were made by Charters (1996) on the basis of the ratios of $C_{16:0}$ and $C_{18:0}$ fatty acids and the carbon-number ranges of intact triacylglycerols present. These identifications have been noted as *Commodities Processed* in Table 1, Appendix 1, p. 333. Four vessels from West Cotton were also submitted to stable carbon isotopic analysis in order to investigate the potential use of this technique in the assignment of origin (Charters, 1996); these results are discussed in Chapter 6.

3.1.1.2 Stanwick (Romano-British/Iron Age)

The samples from Stanwick are part of a larger assemblage screened for organic residues from which 19 out of a total of 210 sherds were selected for further analysis. A detailed account of the results of organic residue analysis of all the sherds from the Stanwick assemblage is given in the English Heritage report on the Raunds Area Project (*Iron Age and Romano-British Project: The Assessments*). In brief, lipid residues (>5 μg g^{-1}

considered significant) were found in >60% of the sherds studied, with degraded animal fats occurring most frequently. In the jar forms analysed, the mean lipid content in rim sherds was 372 μg g^{-1}; in jar body and base sherds, mean lipid content was 96 and 83 μg g^{-1}, respectively. This distribution of lipid accumulation correlated well with the patterns seen in 'cooking' jars from West Cotton. Experimental work has indicated that this distribution reflects the use of these vessel forms for boiling of animal meat/fat, where the lipids float on the surface of the water and are thus more readily absorbed into the rim/shoulder level of the vessel profile (Charters, 1996). Highest concentrations of absorbed lipids were found in jars and dishes, whereas relatively low concentrations have been seen in bowls. This is also consistent with observations by Charters (1996) of vessel use in the later assemblage from nearby West Cotton.

Long-chain alkyl compounds, e.g. alcohols, mid-chain ketones and wax esters (all leaf wax lipids) were detected in numerous vessels, occurring with highest frequency in Mortaria forms, suggesting a bias in their use, e.g. for the tenderising of leafy vegetables, the grinding of herbs, or the production of medicinal preparations (Table 3.1). Mid-chain ketones comprising 31, 33 and 35 carbon atoms, formed during vessel use (Evershed *et al.*, 1995b; Raven *et al.*, 1997), have also been recognised in a number of vessels containing degraded animal fats.

Table 3.1 Occurrence of compounds within different vessel types from Stanwick.

Lipid components	**Vessel type**				
		Roman			***Iron Age***
	Bowls	**Dishes**	**Mortaria**	**Jars**	**Jars**
DAF[1]	8	6	18	8	5
Free fatty acids	4	4	12	6	0
Alcohols	5	6	19	5	2
Sterols	1	1	4	3	0
Wax esters	4	4	18	4	1
Ketones	1	4	3	3	1
No. of whole vessels analysed	12	10	30	14	5

[1] DAF=extracts containing lipid distributions characteristic of degraded animal fats, including free fatty acids, mono-, di- and triacylglycerols.

The extracts selected for further study as part of this project are listed in Table 2, Appendix 1, p. 333. Lipid content ranges between 22 μg g^{-1} and 4 mg g^{-1} dry weight of sherd. Extracts were selected on the basis that they comprised significant abundances of degraded animal fats, although preservation varies between extracts. Particularly well-preserved lipid distributions from degraded animal fats have been seen in Iron Age jars, e.g. the rim sherd from sample 156. Some components derived from leaf waxes have also been identified in these residues, e.g. wax esters, alcohols and the C_{29} ketone are present in samples ST193, ST194, ST215 and ST216. A range of vessels forms including jars, bowls, dishes and Mortaria and types including grey ware, Grogged ware and Shelly ware are represented amongst the samples chosen for further analysis. Other artefacts found in the same contexts as the sherds have also been noted in Table 2, Appendix 1, p. 333.

3.1.2 Sites with an unusually strong bias in the faunal record

3.1.2.1 Fuller's Hill (Late Saxon/early medieval)

The solvent extraction of samples from Fuller's Hill yielded lipid extracts >5 μg g^{-1} in only 2 out of 7 samples analysed, namely GY304 and 349 (Table 3, Appendix 1, p. 333). These samples contained an abundance of saturated $C_{16:0}$ and $C_{18:0}$ fatty acids, characteristic of animal fats. The other extracts yielded unresolved complex mixtures (UCM), comprising an abundance of low molecular weight components eluting between 6 and 16 mins in the HTGC profile shown in Figure 3.1.

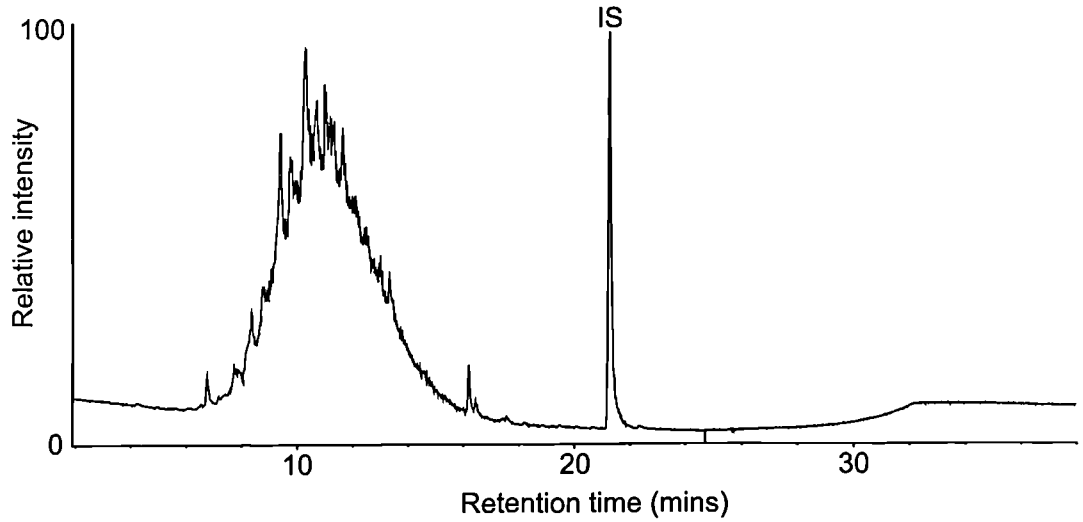


Figure 3.1 A typical example of an unresolved complex mixture (UCM; sample GY197) from the Fuller's Hill assemblage. GC conditions are as for Figure 1.1. IS refers to internal standard.

3.1.2.2 Wicken Bonhunt

Vessels from Wicken Bonhunt were selected from contexts associated with abundant pig bone. The sherds sampled originate mainly from earthenware and Ipswich ware (imported) 'cooking' vessels. The results of the analyses are listed in Table 4, Appendix 1, p. 333. Degraded animal fats comprising free fatty acids, mono-, di- and triacylglycerols were identified in 14 out of the 19 sherds analysed, in which the lipid content ranged between 27 $\mu g\ g^{-1}$ and 1.4 $mg\ g^{-1}$ dry weight of sherd. In all of the samples free fatty acids dominated the lipid extract, indicating that hydrolysis of intact acyl lipid components was well advanced. Mid-chain ketones C_{31}, C_{33} and C_{35} were identified in three of the vessels.

3.1.2.3 Botai, Kazakhstan (early Neolithic)

Six out of the 9 residues analysed (including both absorbed and carbonised residues) contained lipid, ranging between 99 $\mu g\ g^{-1}$ and 2.9 $mg\ g^{-1}$ (Table 5, Appendix 1, p. 333). The lipid distributions seen in extracts from packon III (carbonised surface residue; CR), XVIII and N26 were characteristic of degraded animal fats. The intact triacylglycerols in N26 were found to be remarkably well preserved considering the age of the sherds, however, the lipid distributions in packon I, II (CR) and VII/N21 were comprised predominantly of free fatty acids, indicating that hydrolysis of the original intact acyl lipid components was almost complete in these sherds. No lipid residues were detected in packon I (CR), II or III. Mid-chain ketones, including the C_{31}, C_{33} and C_{35} components (formed according to Evershed *et al.,* 1995b) have been identified in VII/N21 and N26, indicating that the pottery vessels and their contents were strongly heated. The chemical evidence correlates with the physical evidence of sooting and the presence of thick (2-3 mm) carbonised surface residues.

3.1.3 Prehistoric archaeological sites

3.1.3.1 Yarnton Cresswell field (early-middle Iron Age)

Twenty eight of the 49 sherds analysed yielded lipid residues, ranging in abundance between 5 and 411 $\mu g\ g^{-1}$. Preservation of the extracts from Yarnton Cresswell field was extremely variable. Five extracts (samples 101, 108, 117, 138 and 147) comprised only free fatty acid components (Table 6, Appendix 1, p. 333), but excellent preservation of intact triacylglycerols was seen in samples 112, 126, 127, 129, 137 and 146. Mid-chain

ketones were detected in samples 101, 105, 118, 142 and 149. The distributions of lipid components in samples 135 and 142 are characteristic of degraded beeswax (refer to Fig. 1.3). Sample 135 is the body of an unusual, highly decorated vessel, while sample 142 came from a barrel-shaped jar. In the latter, components characteristic of degraded animal fats and mid-chain ketones (C_{31}, C_{33} and C_{35}) were also detected, indicating that this barrel-shaped vessel was utilised in the heating of animal fats and beeswax, perhaps for use in candle making or as fuel for lamps (Hamilakis, 1996). The sherds from the large group of very early Iron Age pottery (*ca* 600-700 BC) at Yarnton Cresswell field contained degraded animal fat residues. The slack-shouldered jar (samples 127, 128 and 129) comprised 44 μg g^{-1} in the base, 15 μg g^{-1} in the body and 142 μg g^{-1} in the rim sherds. Leaf wax components were uncommon in vessels from Cresswell field, with wax esters and alcohols detected only in sample 111.

3.1.3.2 Yarnton flood plain (Neolithic-Bronze Age)

The samples selected from the Yarnton flood plain assemblage comprised 45 sherds from a range of vessel forms and styles, including beakers, biconical urns, Grooved ware and Peterborough ware. Twenty five sherds yielded lipid residues ranging between 5 μg g^{-1} and 358 μg g^{-1} dry weight of sherd (Table 7, Appendix 1, p. 333); thus, almost half of the samples analysed contained no detectable lipid components. Preservation was variable but the majority exhibited lipid distributions characteristic of degraded animal fats, whilst three sherds (samples 32, 33 and 34) comprised free fatty acids alone. Unusually high abundances of monoacylglycerols were noted in the degraded animal fats in vessels 1, 4, 5 and 15, with mid-chain ketones C_{31}, C_{33} and C_{35} present in vessels 5, 21, 24 and 40. Samples 1, 4 and 5 also comprised an abundance of intact acyl lipid components, however, the overall preservation of organic components from the Yarnton flood plain assemblage was poor.

3.1.3.3 Eton Lake End Road (late Neolithic-Early Bronze Age)

Fourteen out of the 24 extracts analysed contained lipid residues >5 μg g^{-1}, with the majority of extracts identified as degraded animal fats and comprising predominantly of free fatty acids (Table 8, Appendix 1, p. 333). A particularly high abundance of free fatty acid (1.2 mg g^{-1}) was detected in sample NRA10. Residues comprising abundant intact

triacylglycerols and similar overall lipid distributions were present in the 4 samples numbered NRA2, all from different parts of the same vessel profile. Mid-chain ketones were present in samples NRA1, 8 (2166), 10 and 13.

3.1.3.4 Eton Rowing Lake (early Neolithic)

Extracts from the Eton Rowing Lake assemblage comprised predominantly free fatty acids, produced through extensive hydrolysis of the intact acyl lipids originally present (Table 9, Appendix 1, p. 333). Sample 13A comprised 1.1 mg g^{-1} of (mainly) free fatty acids. The extent of decay can be seen clearly in the extract from sample 23, a Fengate ware rim sherd, which contained C_{29}-C_{35} mid-chain ketones (Fig. 3.2). The free fatty acids have been completely depleted, whereas the relatively resistant ketones have been preserved, with the C_{31} and C_{35} components still reflecting the original distribution of the $C_{16:0}$ and $C_{18:0}$ free fatty acids, and indicating that the vessel originally contained a range of saturated and straight-chain fatty acid components.

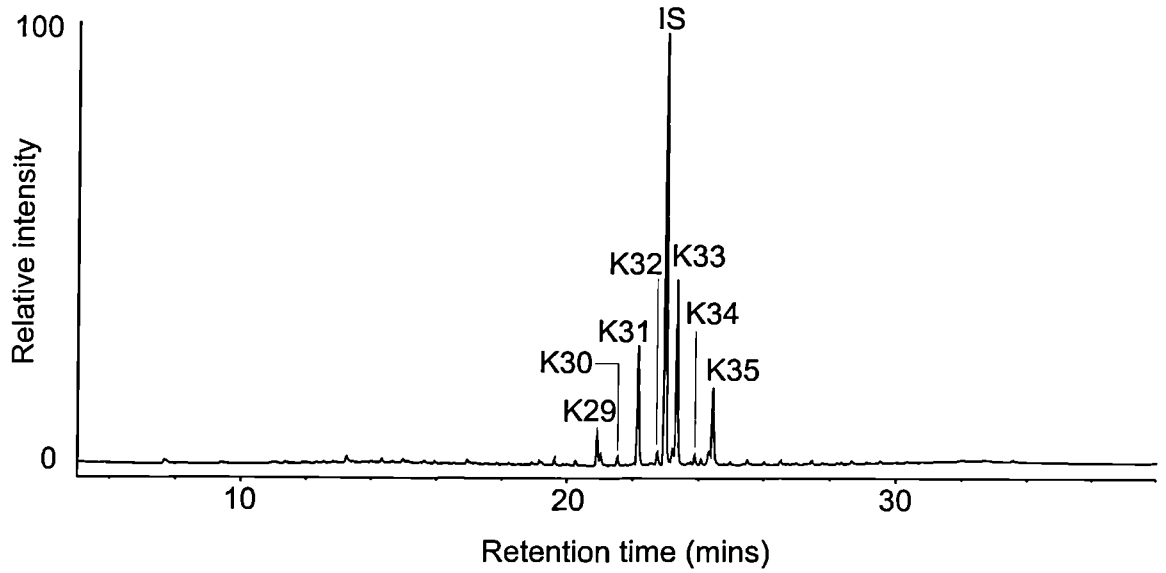


Figure 3.2 Partial HTGC profile of the trimethylsilylated total lipid extract from sample 23, an early Neolithic Fengate ware rim sherd from Eton Rowing Lake, showing the distribution of mid-chain ketones. GC conditions are as for Figure 1.1. K29, K30, K31 etc., refer to mid-chain ketones with 29, 30 and 31 carbon atoms, etc., respectively.

Mid-chain ketones were also detected in samples 7, 8, 11, 13A (Carinated bowls) and 16, 21, 22 and 23 (early Neolithic bowls). Wax ester, alkane and alcohol components, present for example in beeswax and higher plant leaf waxes, were found in abundance in samples

11, 12, 13B, 16 and 19. Figure 3.3 shows the HTGC profile of DBC16, which comprised a relatively well-preserved mixture of degraded animal fat and beeswax. The variety of lipid components which have been preserved is remarkable considering that the sherd was recovered from an early Neolithic context.

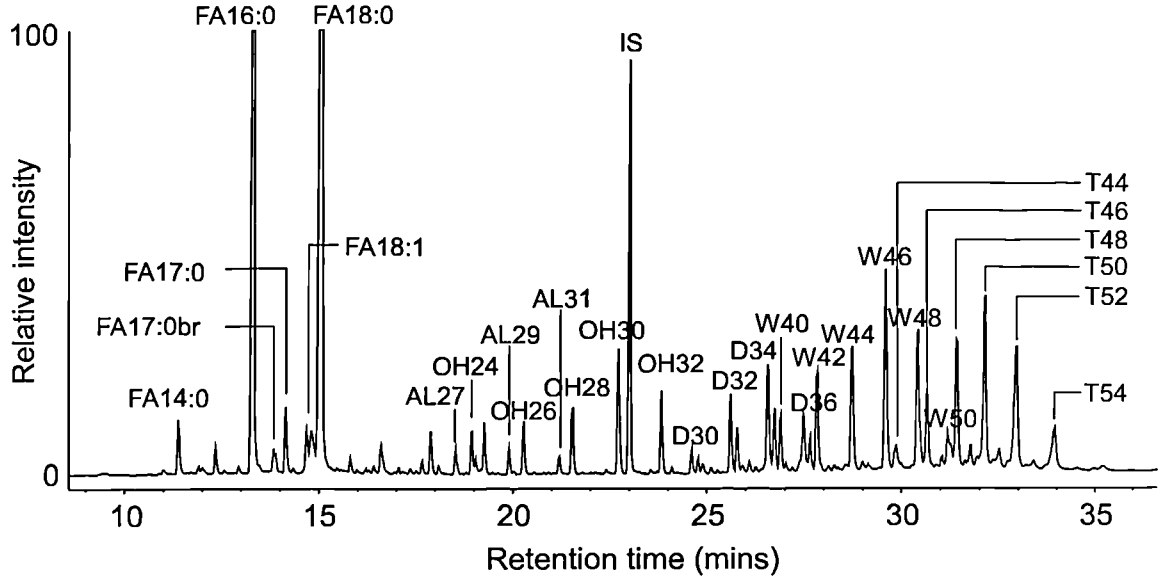


Figure 3.3 Partial HTGC profile of the trimethylsilylated total lipid extract of DBC16, the rim sherd of an early Neolithic bowl. GC conditions and peak identities are as for Figures 1.1, 1.3 and 1.4. In addition, OH24-OH32 refer to primary alcohols with 24-32 carbon atoms, respectively.

3.1.3.5 Upper Ninepence (early-late Neolithic)

Seventeen sherds from both the early and late Neolithic phases were analysed, including sherds from 5 Peterborough ware and 12 Grooved ware vessels. Six of the vessels were covered with thick (*ca* 1-2 mm), black carbonised surface residues which were also screened for lipid components. Ten out of the 17 samples analysed contained amounts of lipid residues considered to be significant (>5 μg g^{-1}; Table 10, Appendix 1, p. 333). Notably, the mean lipid content in absorbed residues from the Peterborough ware was 50% higher (157 μg g^{-1}) than in the Grooved ware (79 μg g^{-1}). In all cases the lipid residues were identified as degraded animal fats, and intact triacylglycerols were detectable in substantial abundances. A degraded animal fat profile typical of the extracts from the carbonised residues from the Grooved ware is shown in Figure 3.4.

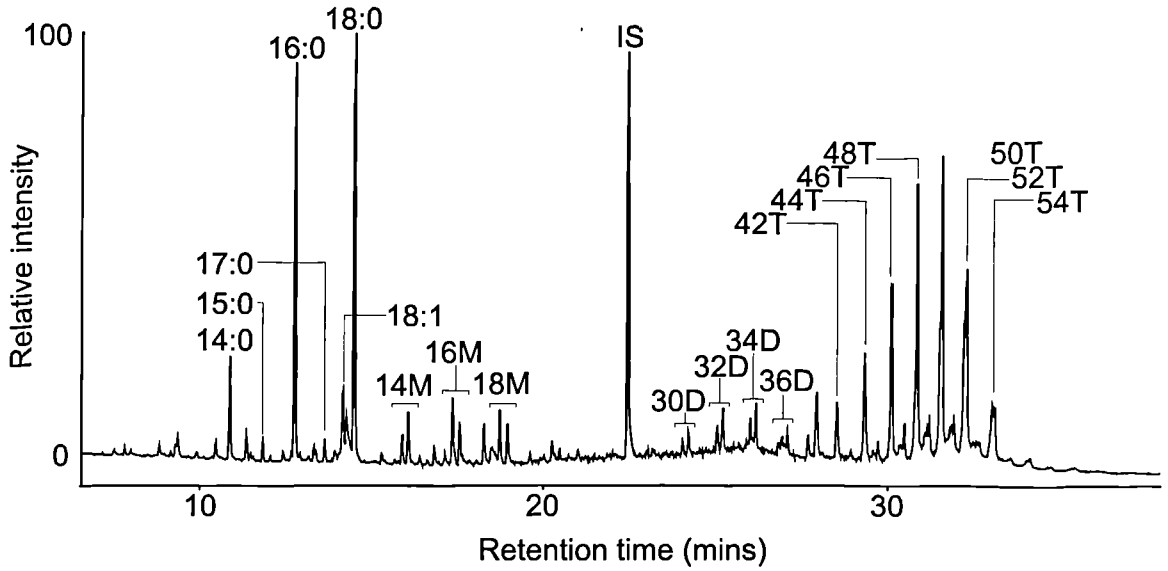


Figure 3.4 Partial HTGC profile of the trimethylsilylated total lipid extract from the carbonised surface residue adhering to P39, a Grooved ware vessel from the Upper Ninepence excavation. GC conditions and peak identities are as for Figure 1.1.

Mid-chain ketones (C_{31}, C_{33} and C_{35}), identifiable as condensation products of acyl lipids formed during the vessel use (Evershed *et al.*, 1995b) have been detected in the three Peterborough ware sherds containing greater than 100 μg g^{-1} of lipid. These components were present in sherds P1a and b and P5 (Table 10, Appendix 1, p. 333). The presence of these components in the Peterborough ware lipid residues indicates that the vessels were held in direct contact with hot embers during use or failure, however significantly, none of these diagnostic mid-chain ketones have been found in the residues associated with the Grooved ware. There was no evidence for the processing of leafy vegetables in any of the samples analysed, although abundant carbonised plant material (cereal grains) have been found in the same archaeological features as the potsherds (Gibson, 1995). Cereal processing would not result in deposits in the vessels of long-chain alkyl components of the class described above.

No correlation has been found between the type of decoration on the surface of the vessels and the presence or absence of absorbed residues. Three sherds from the Grooved ware assemblage, namely P33, P38 and P39, exhibited very low abundances of lipid absorbed in the porous microstructure of the ceramic, but very significant quantities of lipid were preserved in the carbonised residues adhering to the inner walls of the pot. All three vessels were recovered from the same archaeological feature (pit 154) and preservation may have

been influenced by the burial environment. The trace amounts of lipid extracted from the carbonised residues adhering to P1, P21 and P28 may be a function of the degree of carbonisation or the nature of the original deposit, or a combination of the two. Furthermore, none of the sherds from pit 198, including P21 P28, P37 and P48, contained significant lipid residues and both sherds from context no. 133 contained more than 100 μg g^{-1} of lipid. Interestingly, the occurrence of residues appears to correlate with the context from which they were recovered.

3.2 Ethnographic vessels

3.2.1 Vessel A

The initial screening for lipid residues in the tsoukali (vessel A) was carried out by Charters (1996). A second rim sherd has been extracted for use as reference material as part of this study and was found to contain >6 mg g^{-1} of absorbed lipid. The partial gas chromatogram of the total lipid extract from vessel A is shown in Figure 3.5(a). The distribution of lipid components shown is characteristic of a degraded animal fat, comprising a range of free fatty acids, mono-, di- and triacylglycerols. A quite specific distribution of diacylglycerols is noted, comprising only the C_{32}, C_{34} and C_{36} components. The C_{30} diacylglycerol, which is frequently present in degraded animal fat lipid profiles is absent and the C_{34} component is present in relatively high abundance. This distinctive distribution of diacylglycerols may prove to be a diagnostic feature of degraded pork fat. Triacylglycerols ranged from C_{48} to C_{54} (total acyl carbon number) and free fatty acids ranged from C_{14} to C_{18}. No chemical evidence was found for the use of tomato sauce, herbs or seasoning used in the preparation of *yuvetsi* in this vessel. The lipid content and lipid components present in vessels A to G have been tabulated in Table 11, Appendix 1, p. 333.

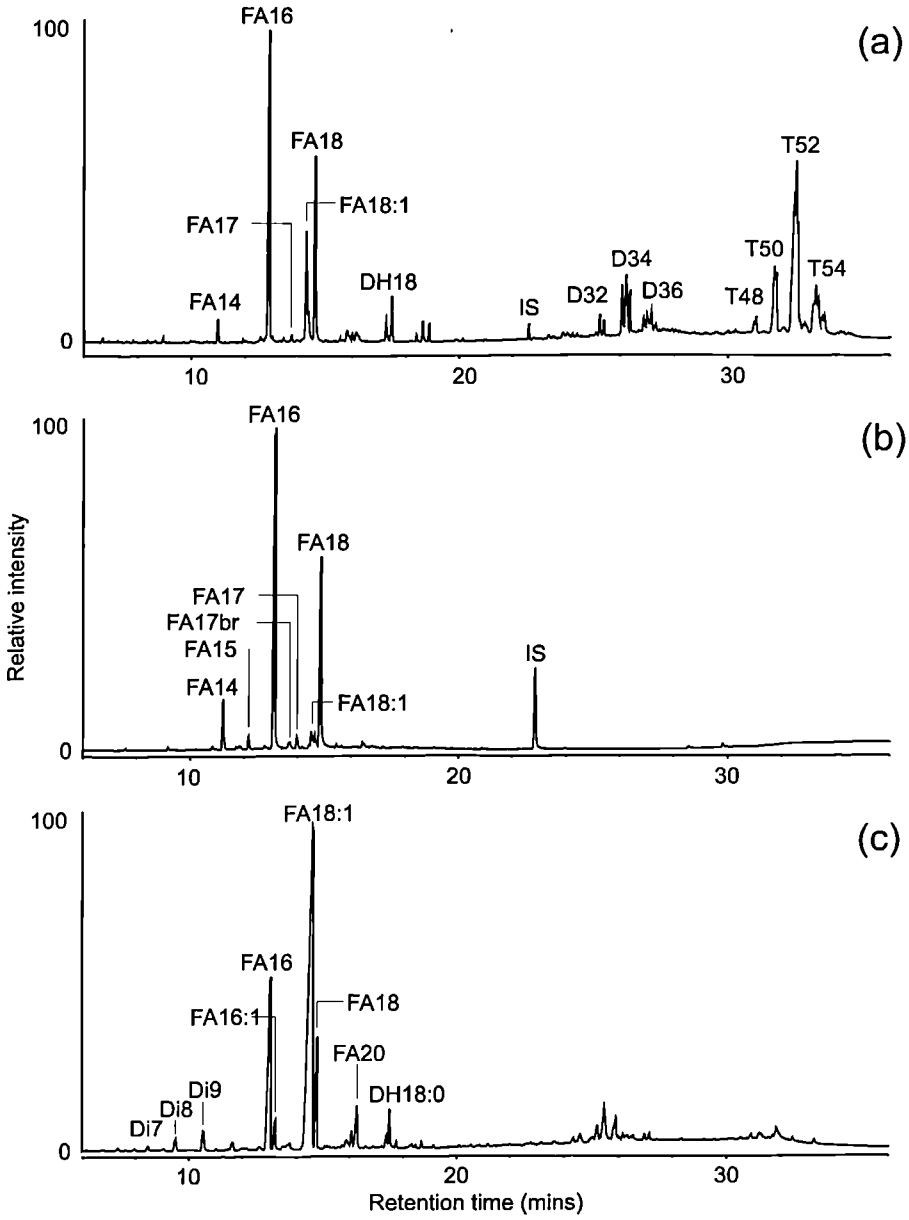


Figure 3.5 Partial HTGC profiles of the trimethylsilylated total lipid extracts from: (a) ethnographic vessel A, a tsoukali type, used for cooking *yuvetsi*, containing pork meat, tomato sauce, oregano, salt and pepper; (b) ethnographic vessel B, a bantia type, used for storing cheese, milk (with yeast and salt) and at other times used to preserve grapes with hardaki (mustard seed), and (c) ethnographic vessel C, used for the storage of olive oil. GC conditions and peak identities are as for Figure 1.1.

3.2.2 Vessel B

The base sherd from vessel B contained only 55 μg g^{-1} of absorbed lipid, comprising free fatty acids [Fig. 3.5(b)]. A low abundance of absorbed lipid was detected in this vessel, perhaps due to the mode of vessel use. Since this vessel functioned as a storage vessel for cheese and milk, the absorption of fat into the vessel wall was not facilitated by heating or

grinding. As in vessel A, chemical evidence was found only for the fat content of the natural products processed and stored in this vessel, with no obvious evidence from the solvent-extractable lipid fraction for the vessel having been used for the preservation of grapes with mustard seed. The diagnostic shorter-chain fatty acid components present in dairy fats were absent, however this is unsurprising due to the extensive decay which has occurred, resulting in the loss of intact acyl lipids.

3.2.3 Vessel C

Vessel C smelled strongly of oil and yielded more than 3 mg g^{-1} of absorbed lipid. The fatty acid profile, including a high abundance of the $C_{18:1}$ fatty acid relative to the $C_{18:0}$ component, is consistent with that of hydrolysed olive oil, however, the intact triacylglycerols are absent. The oxidation of lipid components in this extract is evidenced by the presence of the C_7, C_8 and C_9 diacids and the $C_{18:0}$ dihydroxyacid is also present [Fig. 3.5(c)]. Although hydroxyacids are found in trace amounts in most edible fats, the C_{18} hydroxyacid component in this extract is likely to have arisen mainly through oxidation of the unsaturated C_{18} components in the fat. These components have previously been reported in association with lipid residues in pottery by Regert *et al.* (1998).

3.2.4 Vessel F

A surface scraping from vessel F [Fig. 3.6(a)], derived from the preparation of a pork-containing dish, comprised free fatty acids with a similar distribution to that seen in vessel A, as well as the dihydroxy acid and the diacid oxidation products detected in vessel C. The presence of the dihydroxy acids appears to correlate with residues of the more highly unsaturated fats and oils, i.e. pork and olive oil. Comparison with the pork fat extract from vessel A suggested that this residue has decayed significantly, resulting in the complete loss of the intact triacylglycerols. Despite the extent of the decay, probably due to the fact that the residue was a surface scraping, and not protected by entrapment within the wall of the vessel, the sample analysed comprised more than 10 mg g^{-1} of lipid.

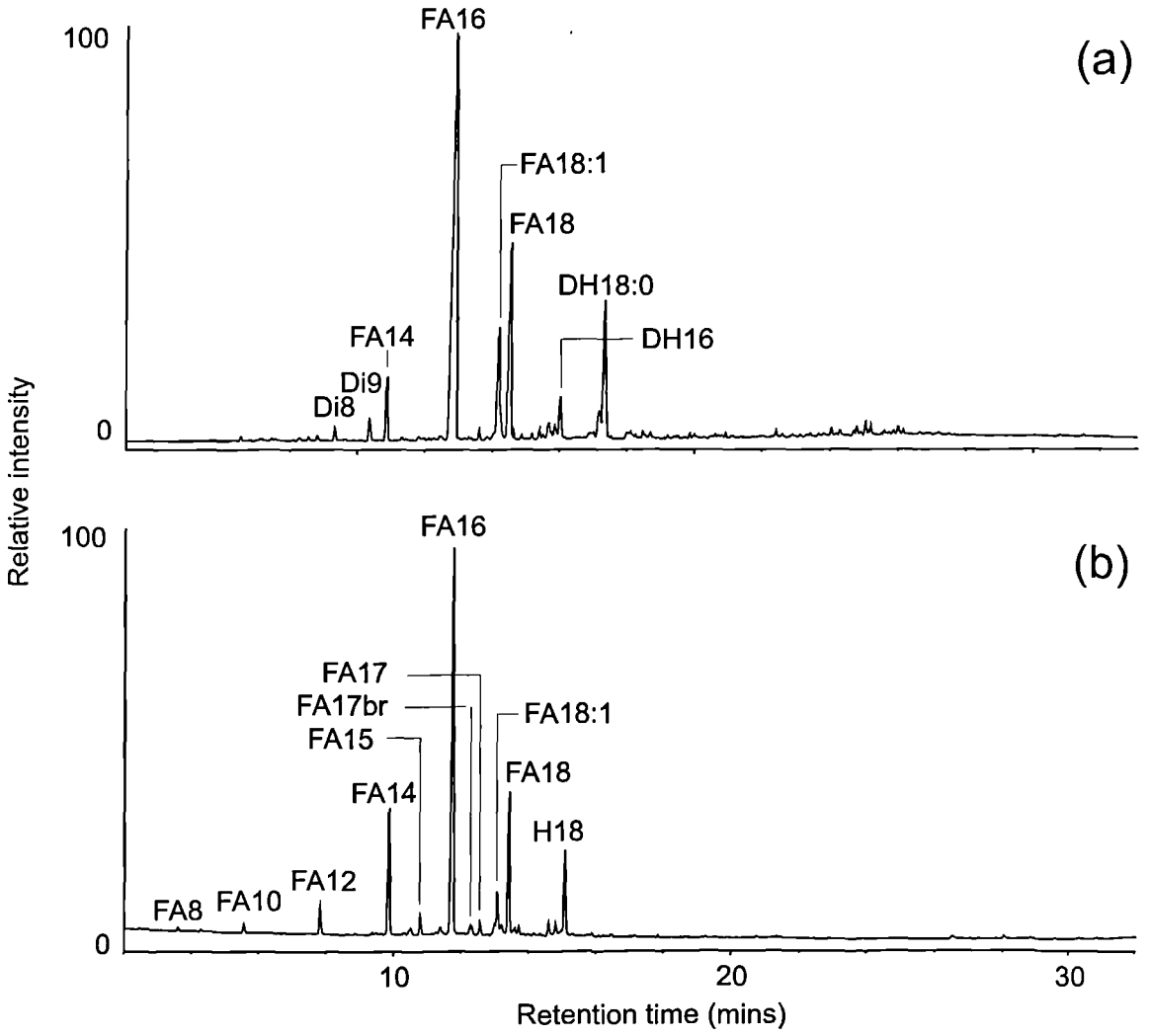


Figure 3.6 Partial HTGC profile of the trimethylsilylated total lipid extracts from: (a) ethnographic vessel F, used for the storage of *kavurmas*, comprising a mixture of pork fat, pork meat, salt, paprika, black pepper, oregano, onions and bahari (spice), and (b) ethnographic vessel G, used for milk and cheese storage. GC conditions and peak identities are as for Figure 1.1. H18 refers to hydroxyoctadecanoic acid; DH16 and DH18 refer to dihydroxyhexadecanoic and -octadecanoic acids, respectively; Di8 and Di9 refer to dicarboxylic acids with 8 and 9 carbon atoms, respectively.

3.2.5 Vessel G

The rim sherd from vessel G contained a low abundance of absorbed lipid (7 $\mu g\ g^{-1}$) comprising free fatty acids alone. Both the ethnographic vessels used in conjunction with milk fats (vessels B and G) comprised a relatively low abundance of lipid compared to those vessels which contained pork fats or olive oil. The cold storage of fats would have resulted in less absorption of lipid than if the vessels and their contents had been heated, e.g. for pasteurising, and furthermore, milk has a lower fat content than pork fat or olive

oil. In vessel G the short-chain fatty acid components diagnostic of milk fat have been preserved and range from C_8 to C_{14} [Fig. 3.6(b)].

3.3 Siberian horse tissues

The sample of stomach lining from Horse 1 comprised free fatty acids and highly abundant hydroxy acids. The degraded fat contained some intact triacylglycerols, however these were present in very low abundance. The lipid components of the stomach contents were also analysed, with the saponified, methylated extract comprising a range of long-chain, saturated and unsaturated free fatty acids. The subcutaneous fat (skin; Horse 1) comprised free fatty acids, hydroxyoctadecanoic acid, cholesterol and a greater abundance of intact triacylglycerols than in the sample of stomach lining from the same horse (Fig. 3.7). The Sacrum meat and crumbled flesh associated with the coccygeal vertebra from Horse 2 yielded an abundance of free fatty acids and also di- and triacylglycerols in low abundance. The lipid components are similar in distribution to the subcutaneous fat from Horse 1. The distributions of free fatty acids, mono-, di- and triacylglycerols in this sample are consistent with other degraded animal fats described and are remarkably well preserved due to the permafrost burial conditions.

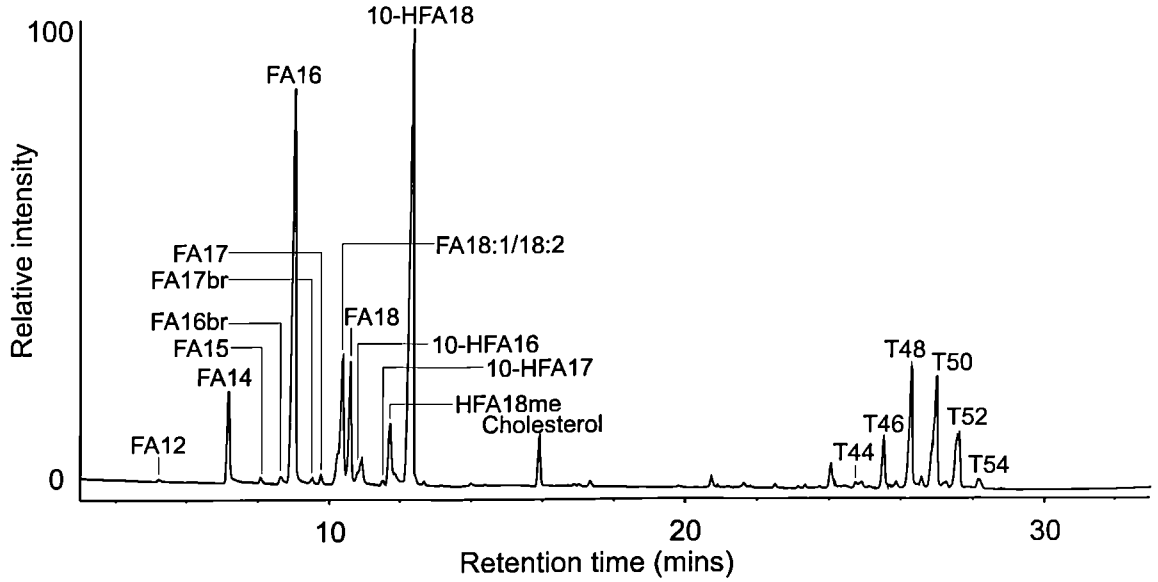


Figure 3.7 Partial HTGC profile of the trimethylsilylated total lipid extract of the remnant fat associated with the horse skin/hide from the 'Ice Princess' burial in the Altay mountains. Peak identities are as in Figure 1.1. In addition, 10-HFA 16, 17 and 18 refer to 10-hydroxy fatty acids with 16, 17 and 18 carbon atoms, respectively; HFA 18 me refers to methyl ester of the C_{18} 10-hydroxy fatty acid.

3.4 Discussion

One of the most unexpected findings from these analyses is the remarkable preservation which has been afforded to the lipid moieties absorbed within the matrix of the ceramic vessels or encapsulated within the carbonised surface residues associated with sherds from early Neolithic vessels. As observed in analyses conducted previously, there has been a high frequency of occurrence of degraded animal fat residues, and no evidence for contamination arising from the migration of lipids from the burial environment. Surprisingly, some of the best-preserved remnant fats from the Stanwick assemblage are actually found in the Iron Age vessels, although sherds from the Late Saxon/early medieval phases at West Cotton and the Romano/British phase at Stanwick yielded a high abundance of lipid, in some cases several mg g^{-1} of powdered sherd. The Neolithic and Bronze Age assemblages generally yielded lower abundances of absorbed lipid, in hundreds rather than thousands of $\mu g\ g^{-1}$. The lower abundances in the latter are most probably reflecting the period of time the vessels have been buried, but may also be related to the methods used to process the animal fats, e.g. roasting or boiling. Table 3.2 compares the number of sherds containing lipid profiles characteristic of remnant animal fats (including free fatty acids, mono-, di- and triacylglycerols) to the number containing only free fatty acids (believed to represent highly degraded animal fats). This ratio gives an indication of the degree of preservation afforded to lipid residues from different archaeological assemblages. The proportion of lipid residues comprising free fatty acids alone, i.e. those which are more highly degraded, varies between sites and is probably related to the burial conditions, since different assemblages from closely situated settlements appear to have undergone similar extents of decay. The degree of preservation is similar between both sites from Eton and is also comparable between the Yarnton sites. Table 3.2 also includes several assemblages analysed as part of other projects in our laboratory for comparison. The advantage of using HTGC in the analysis of total lipid extracts has been exemplified in the detection of abundant intact triacylglycerols in the archaeological fats, enabling the consideration of triacylglycerol distributions in distinguishing between fats of different origins (discussed further in Chapter 5).

Table 3.2 Occurrence of degraded animal fats and free fatty acids in pottery from different archaeological periods

Assemblage	**Date**	**Number of sherds containing:**		**% containing better-preserved fats**	**No. of sherds analysed**	**% containing organic residues**
		DAF[1]	**FFA**[2]			
Walton *Pet. ware*	E Neo	2	2	50	5	80
Eton Rowing Lake	E Neo	16	1	94	29	59
Eton Lake End Road	L Neo/EBA	14	2	88	25	64
Walton *Grooved ware*	L Neo	4	0	100	12	33
Yarnton Flood Plain	Neo/BA	15	4	79	45	42
Yarnton Cresswell field	EIA/MIA	21	6	78	49	55
Stanwick	Iron Age	7	0	100	9	78
Stanwick	Rom-British	63	37	63	122	52
West Cotton	Saxon/Med	34	10	77	74[3]	59
Wicken Bonhunt	M Saxon	14	0	100	19	74
Barking Abbey	M Saxon	14	2	88	30	53
Brandon	M Saxon	26	13	67	56	70
Canterbury	M Saxon	15	2	88	30	57
Flixborough	M Saxon	10	8	56	30	60
Ipswich	M Saxon	25	5	83	54	56
North Raunds	M Saxon	8	16	33	30	80

[1] Components characteristic of degraded animal fats, including free fatty acids, mono-, di- and triacylglycerols

[2] Free fatty acids alone

[3] Total number of vessels analysed

The West Cotton vessels all derive from a domestic assemblage, and the range of commodities recognised from their lipid profiles is diverse, with evidence for the processing of leafy vegetables, beeswax, animal products and also mixtures of animal and plant materials. Since detailed descriptions of the sherds and context information are available for both the West Cotton and Stanwick assemblages we hope to be able to relate remnant fats of different origins to certain vessel types and investigate whether particular animal products were processed alone or in conjunction with other commodities, i.e. leafy vegetables. There maybe a higher plant contribution to the TLE of some sherds, although

this is anticipated to be minimal and should not distort the dominant chemical characteristics of the animal fats. The wax ester and other components derived from higher plants are less frequent in the Stanwick sherds with a higher proportion of the extracts representing pure animal fat signals. The detailed accounts of the faunal remains from these sites will enable the animal species identified in pottery residues to be compared with the major domesticated species represented in the faunal assemblages. It is anticipated that the majority of remnant fats should derive from the major domesticates identified in the faunal remains from West Cotton and Stanwick, i.e. cows, sheep and pigs, however biases may arise in the types of animal products processed in pottery vessels due to different methods of processing, e.g. spit roasting, frying on hot stones, boiling in vessels etc. Indeed, in a recent paper it has been suggested that non-ruminant fats found in medieval 'dripping' dishes derive from the fat falling from spit roasting animals (Evershed *et al.*, 1997a; Mottram *et al.*, 1999), perhaps indicating that residues of porcine fats are less likely to be found in 'cooking' pots.

It will also be interesting to compare the characteristics of remnant fats from the assemblages from Yarnton flood plain and Yarnton Cresswell field, and to note any differences which may relate to the transition of settlement from the Late Bronze Age to the early Iron Age. The latter is thought to be associated with greater permanence of settlement, a shift to arable agriculture and more intensive exploitation of the land. Initial observations indicate that lipid residues from both Yarnton flood plain and Yarnton Cresswell field are comparable, both assemblages comprising remnant animal fat residues in a similar state of preservation. However, two distinctive vessels from Yarnton Cresswell field were found to contain degraded beeswax residues which were not detected in sherds from the earlier Neolithic-Bronze Age phase. Residues from the early Neolithic Eton assemblage were distinguished by the more frequent occurrence of long-chain alcohols and wax esters, possibly derived from beeswax or leaf waxes. Degraded animal fats were predominant in vessels from the late Neolithic-Early Bronze Age site at Eton.

Hydroxy acids have been detected in abundance in both the archaeological horse fats and solvent extracts of the ethnographic vessels. Hydroxyoctadecanoic acid is considered a marker for adipocere and has also been seen in abundance in samples of 'bog butter' from

lowland regions of Scotland (Lawrence, 1994; Berstan, 1996), in skin samples of bog bodies (Evershed, 1990) and in the 'Ice man' discovered near the Tiesenjoch, Austria (Mayer *et al.*, 1997). In the latter, hydroxyoctadecanoic acid comprised 97% of the total fatty acid. β-hydroxy fatty acids are known to be produced from β-oxidation of saturated fatty acids, and similarly, ω-oxidation of saturated fatty acids to ω-hydroxy acids by microbial oxidation has been reported (Killops and Killops, 1993). ω-Hydroxy acids are known to be more resistant to decay than unsaturated acyclic fatty acids (both microbially and chemically) and once formed are considered relatively stable which may be why they are preserved in such abundance in the archaeological and ethnographic fats. It has been suggested by den Dooren de Jong (1961) that the hydroxy acid in adipocere forms first by hydrogenation of $C_{18:1}$ to $C_{18:0}$ before dehydrogenation. A mechanism proposed by Schnoepper (1966) states that the double bonds of unsaturated fatty acids are hydrated under certain reductive conditions, however, this does not explain why hydroxy acids are detected predominantly in the remains of the highly unsaturated fats and oils studied herein, which suggests that they derive directly from the unsaturated fatty acid.

It is highly probable that oxidation has also played a role in the formation of the UCM detected in the Fuller's Hill extracts. It is interesting in itself that UCMs occur with such high frequency in the Fuller's Hill assemblage. If these residues represent degraded fish oils, hydrolysis and polymerisation during processing or burial may account for the presence of highly complex mixtures of lipid components in the extracts which are unresolvable by GC. Degradative oxidation reactions can lead to the production of low molecular weight degradation products, e.g. through hydroperoxide intermediates (Mills and White, 1994; see Section 7.1.3 for further explanation), and conjugated fatty acids are highly susceptible to polymerisation, e.g. linked by C-C bonds (Davídek *et al.*, 1990).

Remnant fats from Wicken Bonhunt will be compared with the degraded pork fats from the two ethnographic vessels and with the modern reference pig fats in order to identify the chemical characteristics of porcine fats which can be used to distinguish similar fats in other archaeological assemblages. Similarly, the results of the laboratory decay of lamb fat conducted by Charters (1996), the remnant milk fats and olive oil in the ethnographic vessels and from the laboratory decay experiments described in Chapter 7, and the inherent

characteristics of the other modern reference fats will be compared with the archaeological fats. The viability of comparing the Siberian horse fats with remnant fats recovered from the Botai potsherds and with the fats from modern animals bred in the UK will also be investigated, and the overall reliability of different criteria in drawing distinctions between remnant fats will be determined.

CHAPTER 4
Fatty acid compositions

4.1 Variation in depot fat fatty acid composition between species

Fatty acids are the building blocks of the most abundant storage lipids in the animal kingdom. The accepted pathway for the *de novo* formation of fatty acids in general has been discussed in Section 1.6.3. Their exact nature and distribution in animal body fats varies considerably and is dependant upon factors such as dietary fatty acid intake, tissue location and age as well as animal species, physiology and metabolism (Section 1.8).

Perhaps the most significant differences in the distribution of fatty acids comprising depot fats can be seen between ruminant and non-ruminant animal fats. This is essentially due to the extremely complex metabolic origin of ruminant fatty acids (Harfoot, 1978; Noble, 1978). As a result of microbial breakdown processes in the rumen, the body tissues of ruminants will contain higher levels of saturated fatty acids, *cis*- and *trans*-monoenoic acids (including positional isomers) and branched-chain fatty acids than non-ruminant fats. The appearance of $C_{14:1}$ *cis*-Δ^{13}, $C_{16:1}$ *cis*-Δ^{11}, $C_{17:1}$ *cis*-Δ^{10} and $C_{18:1}$ *cis*-Δ^{9} acids can be related to the activity of direct Δ^{9}-desaturase activities on the respective saturated moieties (Hay and Morrison, 1970, 1973). $C_{17:1}$ *cis*-Δ^{10} may be produced by α-oxidation of the $C_{18:1}$ *cis*-Δ^{9} fatty acid and a high proportion of the $C_{16:1}$ *trans*-isomers, $C_{16:1}$ *cis*-Δ^{11} and $C_{14:1}$ *cis*-Δ^{11}, could arise by β-oxidation of the $C_{18:1}$ *trans*-isomers, $C_{18:1}$ *cis*-Δ^{9} and $C_{16:1}$ *cis*-Δ^{9}, respectively (Harfoot, 1978; Christie, 1978; Hay and Morrison, 1970, 1973). Although there is considerable variation between fatty acid distributions in different species, this is minimal within species and particularly within the same fat type, e.g. depot fat. Therefore, consideration of the fatty acid distributions in animal depot fats may enable distinctions to be drawn between animals of different classes.

4.1.1 Animal fat compositions in modern domesticates

Extensive reports can be found in the literature regarding lipid distributions in modern animal fats (e.g. Christie, 1978; Body, 1988; Enser, 1991; Gunstone *et al.*, 1986), and a synopsis of the published data giving distributions of the major fatty acid components in different fats is given in Tables 1 to 10, Appendix 2 (pp. 353-358).

4.1.1.1 Ruminant fats

The major fatty acids in cow adipose are $C_{16:0}$ and $C_{18:1}$, with a lower proportion of $C_{18:0}$. Analysis of subcutaneous and perirenal fat has also revealed a range of branched-chain components (Spencer *et al.*, 1976) and a number of positional isomers of the $C_{18:1}$ component are also known to occur where the $C_{18:1}$ Δ^9 was found to be the major *cis*-monoenoic acid, with a much smaller amount of palmitoleic acid (Harfoot, 1978). The $C_{18:1}$ Δ^{11} was the most abundant *trans* acid, followed by the Δ^{10}.

Sheep fat is similar to cow fat in composition, predominantly composed of C_{16}, C_{18} and $C_{18:1}$ fatty acids and comprising a range of branched-chain and odd-carbon number components, although sheep fat is slightly lower in $C_{16:0}$ and $C_{16:1}$ and slightly higher in linolenic ($C_{18:3}$) and linoleic ($C_{18:2}$; Whitehead and Turrel, 1988). Proportions of *cis*- and *trans*-isomers of the $C_{18:1}$ component have been reported by Christie and Moore (1971); perirenal fat comprised 27.2% *cis*- (moles %) and 4.2% *trans*-$C_{18:1}$; omental fat comprised 29.7% *cis*- and 4.4% *trans*-$C_{18:1}$, while subcutaneous (chest) fat comprised 33.7% *cis*- and 4.2% *trans*-$C_{18:1}$.

Deer subcutaneous fat comprises primarily $C_{16:0}$ and $C_{18:0}$ with lesser amounts of $C_{18:1}$ (Shorland *et al.*, 1952). In red deer (*Cervus elephus*; Garton and Duncan, 1971;) and white tailed deer (*Odocoileus virginianus*; Garton *et al.*, 1971) the content of unusual fatty acids, particularly *trans*-unsaturated components and $C_{18:2}$ acid, was found to be low. This may reflect the diet of some of these species, i.e. lichens, mosses, heather, twigs and dried leaves, which would contain lower amounts of polyunsaturated fatty acids than fresh herbage. Positional isomer studies showed perinephric fat from red deer is comprised of 14.3 (moles %) *cis*- and 0.9% *trans*-$C_{18:1}$; while omental fat comprised 13.0% *cis*- and 0.3% *trans*-$C_{18:1}$ and subcutaneous fat comprised 18.1% *cis*- and 0.6% *trans*-$C_{18:1}$ (Christie and Moore, 1971).

Milk fatty acids are numerous and include aliphatic acids from C_2 to C_{28}, mono-methyl branched-chain fatty acids from C_{11} to C_{28} (including numerous positional isomers), multimethyl branched-chain fatty acids from C_{16} to C_{28}, *cis*- and *trans*-monoenoic acids from C_{10} to C_{26}, numerous di- and polyenoic fatty acids, keto and hydroxy fatty acids and

cyclohexyl fatty acids (Patton and Jensen, 1975). Milk fats from herbivores contain diagnostic short-chain fatty acids (ranging from C_2 to C_{14}) with abundant butyric acid. The lipid composition of milk changes according to the stage of lactation, with the first milk (colostrum) containing relatively high amounts of fat and protein. Published data on positional isomers in milk fat are scarce, however, the main *trans*-monounsaturated acid in milk fat is reported to be *trans*-vaccenic acid ($C_{18:1}$ Δ^{11}; Hay and Morrison, 1970; Lund and Jensen, 1983). The complexity of milk fatty acids arises from a number of factors including: (i) the synthesis of short chain fatty acids (C_4 to C_{10}) within the mammary gland, and (ii) micro-organisms in the rumen which fully or partially hydrogenate polyunsaturated C_{18} fatty acids in the diet, thereby introducing geometric and positional monounsaturated isomers.

4.1.1.2 Non-ruminant fats

As discussed in Chapter 1, the adipose tissue of horses and other monogastric grazing animals reflects the incorporation of dietary fatty acids from vegetation, such as $C_{18:2}$ and $C_{18:3}$ (Payne, 1971). Similarly, omnivores accumulate $C_{18:2}$ from the diet directly into their adipose tissue, e.g. human fat contains branched-chain components from milk and ruminant fat in the diet (Jacob and Grimmer, 1967; Shorland, *et al.*, 1969). The more unusual branched-chain and *trans*-fatty acids would be expected to be less abundant than in ruminant fats due to the lower activity of microbial action in the gut, however, branched-chain and odd-carbon number fatty acids may be incorporated into adipose fats if they are present in the diet (see Section 1.8.5).

Horse body fats contain relatively high amounts of $C_{18:3}$. They consist of a complicated mixture of mixed glycerides, the chief components comprising about 30% $C_{16:0}/C_{18:1}/C_{18:3}$ and 19% triunsaturated glycerides with one $C_{18:1}$. The rest is comprised of about 7% each of the $C_{16:0}/C_{16:0}/C_{18:1}$, $C_{18:1}/C_{14:0}/C_{16:0}$ and $C_{18:1}/C_{16:0}/C_{18:0}$. Five percent is comprised of $C_{16:0}/C_{18:1}/C_{18:1}$; $C_{16:0}/C_{16:1}/C_{18:1}$, $C_{16:0}/C_{18:1}/C_{18:2}$ and about 2-3% of mixed saturated triglycerides. Fats from pasture-reared horses analysed by Shorland *et al.* (1952) comprised 2.4% $C_{14:0}$, 29.7% $C_{16:0}$, 4.3% $C_{18:0}$ and 32.5% unsaturated C_{18}. Published data are sparse because these animals are not as commercially important as the major domesticates.

Pork is more highly unsaturated than the other animal fats, the major fatty acids being $C_{16:0}$, $C_{18:1}$ and $C_{18:0}$, with relatively high levels of $C_{18:2}$ and eicosadienoic ($C_{20:2}$) acid. The higher amounts of $C_{18:1}$ and lower amounts of $C_{18:0}$ than ruminant tallow give pork fat its softness (Spencer *et al.*, 1976; Hubbard and Pocklington, 1968). Minor differences in composition have been reported between outer subcutaneous fats from shoulder, loin and rump and belly sites in pigs (St John *et al.*, 1987; Jeremiah, 1982; Sink *et al.*, 1964), however, perirenal fats have been shown to comprise higher proportions of saturated fatty acids (Christensen, 1963) and lower proportions of unsaturated fatty acids (Koch *et al.*, 1968; Sink *et al.*, 1964) than subcutaneous fats.

Hen body fats are differentiated from the depot fats of pigs, sheep and cows by their unusually high abundances of unsaturated C_{18} triacylglycerols. Total fatty acids comprise 27.1% $C_{16:0}$, 6.7% $C_{18:0}$ and 36.2% unsaturated C_{18} (Hilditch and Stainsby, 1935). Fully saturated triglycerides formed only 2.5% of the fat (mainly tripalmitin with some palmitostearin).

Fish oils are generally more unsaturated than mammalian fats; polyunsaturated fatty acids dominate and are most characteristic of fish oils and indeed marine oils in general. The major saturated fatty acids are $C_{14:0}$ and $C_{16:0}$. The monounsaturated fatty acids are composed mainly of palmitoleic acid ($C_{16:1}$ Δ^{11}), oleic acid ($C_{18:1}$ Δ^{9}) and its isomer *cis*-vaccenic acid ($C_{18:1}$ Δ^{11}). These are normally accompanied by large amounts of $C_{20:1}$ Δ^{9} and $C_{22:1}$ Δ^{9}. Data on positional isomers of the $C_{18:1}$ fatty acid in fish oil have been reported by Ackman (1980), including Mackerel of which the $C_{18:1}$ Δ^{9} comprises 8.6%, Δ^{11}, 3.8% and Δ^{13}, 0.5%, and Atlantic Herring in which $C_{18:1}$ Δ^{9} comprises 12.3%, Δ^{11}, 3.7% and Δ^{13}, 0.6%. $C_{18:2}$ and $C_{18:3}$ acids commonly found in vegetable oils occur to no more than 1 or 2% in fish oils. The major polyenoic acids are usually $C_{20:5}$ (Δ^{15}) and $C_{22:6}$ (Δ^{15}). Branched-chain fatty acids are present in low abundance, and almost without exception, fish fatty acids contain even numbers of carbon atoms. Although fatty acids can also be synthesised *de novo*, the wide variety of fatty acids present are absorbed directly from the diet. The acids with five and six double bonds originate in unicellular phytoplankton and in seaweeds.

4.1.2 Previous work to distinguish between processed, mixed and degraded animal fats

Differences between fresh animal fats, e.g. milk, raw and cooked meats and cheese, have been demonstrated based upon the distributions of fatty acids obtained by GC analysis of transesterified triacylglycerols (Matter, 1992; Matter *et al.*, 1989). Distinctions were based on abundances of minor components, including $C_{14:1}$ in sheep milk and $C_{20:2}$ in pork, and for mixtures of fats the technique was unsuccessful. Unsaturated components, such as $C_{14:1}$, although diagnostic in fresh fats, are unlikely to be detectable in degraded archaeological fats due to their relatively high susceptibility to oxidation (Mills and White, 1994).

Characterisations of archaeological fats based upon fatty acid ratios have been attempted previously, with one of the earliest and largest studies by Rottländer (1990). Rottländer succeeded in recovering substantial amounts of fatty acids from visible black (carbonised) surface deposits on the interior of unglazed pottery vessels and made tentative characterisations of the different residues based upon the distributions of saturated fatty acids present, particularly $C_{16:0}$ and $C_{18:0}$. These assignments were flawed in that fatty acid distributions alone were not enough to provide unambiguous evidence for a particular animal fat or plant oil origin, partly due to the loss of the more diagnostic mono- and polyunsaturated fatty acid components during processing and/or burial. In an in-depth investigation of absorbed residues from pottery vessels from West Cotton, Northamptonshire, Charters (1996) assigned origins to remnant fats based upon the ratios of the $C_{16:0}$ and $C_{18:0}$ fatty acids in the total lipid extracts. Comparisons were made with the ratios of fatty acids in reference ruminant and non-ruminant fats reported by Mills and White (1994). The remnant fats were tentatively identified as either mutton, beef, pork or mixtures of mutton and beef fat.

4.1.3 Decay and transformations of fatty acids

The major limiting factor in the use of fatty acid distributions for distinguishing between archaeological fats of different origin is that of decay. Although lipids are generally considered to be relatively resistant to decay compared to other biochemical fractions (Eglinton and Logan, 1991), fatty acids with one or more double bonds are known to be readily degraded under certain conditions, with polyunsaturated fatty acids the least resistant (Mills and White, 1994). Polyunsaturated fatty acids such as $C_{18:3}$ derive their

reactivity from the isolated methylene group between the double bonds and are much less likely to survive on archaeological time scales (Frankel, 1980), whereas oleic acid, having only one double bond, is relatively unreactive. The oxidative crosslinking of unsaturated fatty acids has been shown to form oligomeric mixtures during drying (Muizebelt and Nielen, 1996 and references therein), and furthermore, shorter-chain length and polyunsaturated fatty acids are more water soluble than their longer-chain, saturated counterparts (Gunstone *et al.*, 1986). These factors are likely to have serious consequences for the reliable use of fatty acid distributions in characterising degraded animal fats.

The configuration of the double bonds in fatty acids also affects the stability of the molecule. Unsaturated fatty acids of higher plants and animals are comprised mainly of double bonds in the *cis*-configuration which infers rigidity in the hydrocarbon chain. In the *trans*-isomer the methylene groups are well separated, whereas in the *cis*-isomer they are crowded together and thus the *cis*-isomer experiences greater steric repulsion which destabilises the molecule, and consequently the *cis* form is under greater strain than the *trans*. The lower hydrogenation and also combustion heat of the *trans*-isomer indicates its higher stability (Morrison and Boyd, 1987). Double bonds can be hydrogenated with considerable ease in the presence of a metal catalyst, e.g. nickel, the rate of hydrogenation dependant upon factors such as reaction temperature (optimum is ca. 200°C), and proportion of catalyst. The hydrogenation reaction is accompanied by shifts in the double bond positions and by *cis-trans* isomerisation (Patterson, 1983). Although these conditions are unlikely to be met in nature, *trans*-unsaturated fatty acids are known to be formed without a catalyst, simply by intense heating (Davídek *et al.*, 1990). Scrambling of the double bond position during decay has been reported in monoenoic fatty acids present in human adipocere (Evershed, 1991).

In this chapter the distributions of the major fatty acids in depot and dairy fats from C_3-raised animals have been determined and compared with the distributions seen in remnant fats from the archaeological assemblages and ethnographic vessels. Furthermore, the positional distributions and geometric configurations of $C_{18:1}$ fatty acid isomers have been quantified for both modern reference and remnant fats to determine their effectiveness in identifying species origin.

4.2 Fatty acid compositions and $C_{18:1}$ positional and geometric isomers of fats from modern C_3-raised animals and reference oils

4.2.1 Analysis of reference fats and oils

Reference fats and oils were solvent extracted (Section 9.1.2), saponified (Section 9.1.5) and methylated (Section 9.1.6) prior to GC analysis (Section 9.2.1). A typical example of the resolution obtained on the 50m CP-wax-52 CB capillary column used in the analyses is shown in Figure 4.1. The branched-chain $C_{17:0}$ *iso*- and *anteiso*-fatty acids and the $C_{18:0}$, $C_{18:1}$, $C_{18:2}$ and $C_{18:3}$ fatty acids are clearly resolved. Compound identifications were made by GC/MS analysis (Section 9.2.2) and quantification of fatty acid methyl esters (FAME) was carried out using electronic integration of peak areas.

Positional isomer analysis of the $C_{18:1}$ fatty acids in the reference materials was carried out by GC/MS analysis of their dimethyl disulphide (DMDS) derivatives (Section 9.1.7). Quantification was carried out manually using relative abundances of the major fragment ions. In some cases the $C_{18:1}$ *trans*-Δ^9 isomer proved difficult to quantify due to the overwhelming abundance of the corresponding *cis*-isomer. Data obtained for the reference materials are shown in Tables 1 and 2, Appendix 4 (pp. 374-375).

The relative abundance distributions of the major fatty acids in C_3-reared reference animal fats and reference oils described in Chapter 2 are given in Appendix 3 (pp. 359-373). Mean fatty acid abundances in individual species are shown in Figures 4.2 and 4.9. The relative abundances of the saturated fatty acids in animal fats are of greatest interest since these are the most likely to survive over archaeological time.

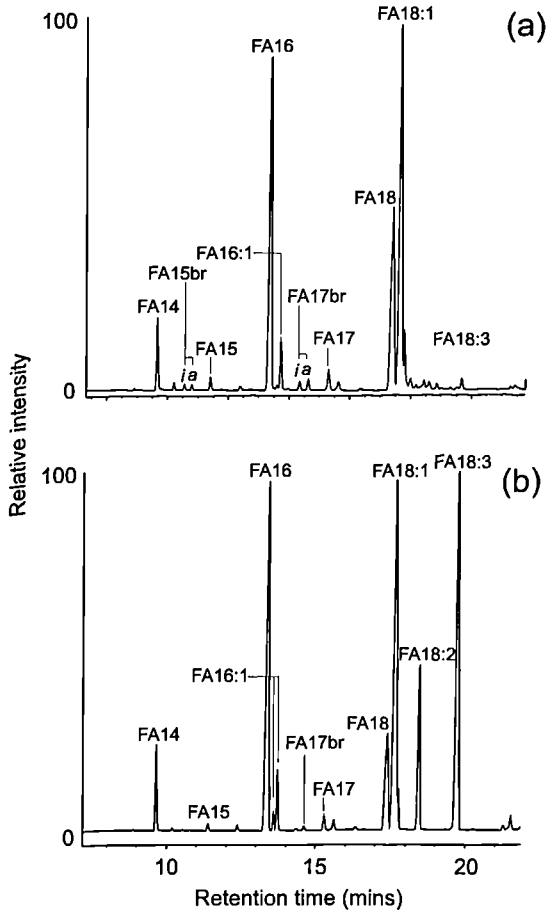


Figure 4.1 Partial gas chromatogram showing the separation of FAMEs from: (a) reference cattle adipose (sample C1BB) and (b) reference horse adipose (sample H5PP). The analyses were performed on a 25 m x 0.32 mm i.d. WCOT fused silica capillary, coated with CP-Wax-52 CB stationary phase (polyethylene glycol, 0.2 μm film thickness). The temperature programme consisted of two ramps from 40 to 220°C at 4°C min^{-1} and from 220 to 240°C at 15°C min^{-1} remaining at 240°C for 15 min and hydrogen was used as carrier gas. Sample introduction was by on-column injection. Peak identities are as in Figure 1.1. In addition, *i* and *a* refer to *iso*- and *anteiso*-branched-chain fatty acids, respectively.

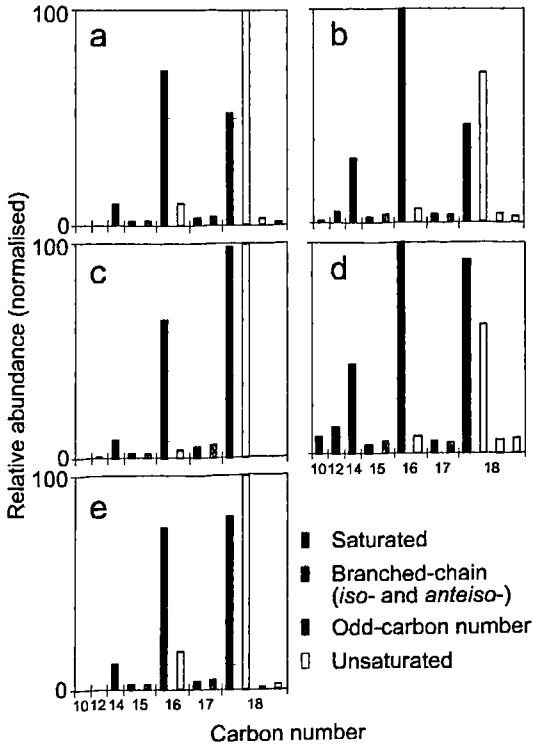


Figure 4.2 Relative abundances of fatty acids in reference ruminant animal fats: (a) cows' adipose (n=4); (b) cows' milk (n=8); (c) sheep adipose (n=13); (d) sheep milk (n=2), and (e) deer adipose (n=7).

4.2.2 Bovine fats

4.2.2.1 Cow adipose

$C_{16:0}$, $C_{18:0}$ and $C_{18:1}$ fatty acids are the major components in the reference cow adipose fats, with minor amounts of polyunsaturated C_{18} fatty acids and an absence of fatty acids of carbon number less than C_{14}. There is a greater abundance of $C_{16:0}$ than $C_{18:0}$ (ratio 1.4:1), and branched-chain and odd-carbon number components, including aliphatic and *iso*- and *anteiso*-$C_{15:0}$ and $C_{17:0}$ fatty acids are present in low abundance. Figure 4.2(a) shows the ratios of the saturated and unsaturated fatty acids in cow adipose (mean values) and Figure 4.3 compares the relative abundances of $C_{16:0}$ and $C_{18:0}$ fatty acids compared with other animal fats. The ratios of the $C_{14:0}$ and $C_{17:0}$ fatty acids are given in Table 23, Appendix 3 (pp. 369-370). $C_{14:0}/C_{17:0}$ ratios are significantly lower in the cows' adipose (mean=2.7) than in the cows' milk (mean=9.9). The abundances of branched-chain and straight-chain $C_{17:0}$ components are approximately equal in cow adipose, although there was some variation between individual animals. Differences were seen in the % abundance of saturated fatty acids between fat samples from different depot fat locations within the

same animal, with $C_{16:0}$ ranging between 27-30%, $C_{18:0}$ 12-32% and $C_{18:1}$ 30-48%, hence, the greatest variations being in the proportions of different C_{18} components.

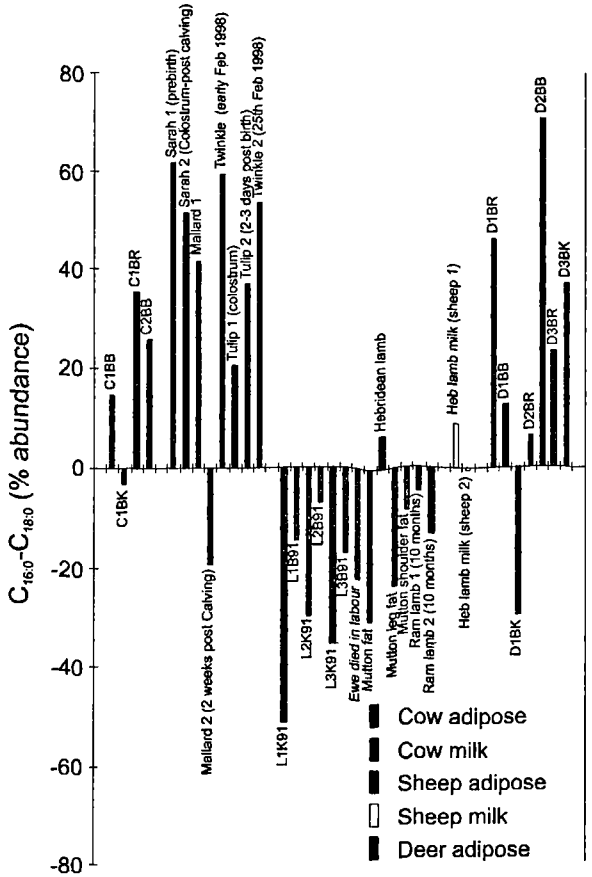


Figure 4.3 Relative abundances of $C_{16:0}$ and $C_{18:0}$ fatty acids in reference ruminant animal fats. The % abundance of the $C_{16:0}$ fatty acid has been subtracted from the % abundance of the $C_{18:0}$ fatty acid to give positive values to samples comprising a greater abundance of the $C_{16:0}$ fatty acid and *vice versa.*

The distributions of positional and geometric isomers of the C_{18} component in cow adipose are shown in Figure 4.4. The Δ^9 to Δ^{15} isomers (inclusive) have been quantified and *cis*- and *trans*-isomers have been indicated in blue and green (filled bars), respectively. Cow depot fat, as in all ruminant fats, contains an abundance of positional isomers, including all the *cis*- and *trans*-isomers from Δ^9 to Δ^{15}. The *cis*-Δ^9 is by far the most abundant isomer, comprising 85-87% (Figure 4.4; Note: the scale has been expanded to show the relative abundances of other isomers present). The second most abundant isomers are the *cis*- and *trans*-Δ^{11}. In general the Δ^{12}, Δ^{13}, Δ^{14} and Δ^{15} *trans*-isomers are more abundant than their *cis*-isomers. The ratio of *trans*-Δ^{11}/*trans*-Δ^{10}-isomers and *cis*-Δ^{11}/ *cis*-Δ^{10}-isomers in the

cow adipose samples analysed are shown in Figure 4.5. The difference between the *trans*-Δ^{11} and the *trans*-Δ^{10} (green diamond) is greater than the difference between the *cis*-Δ^{11} and the *cis*-Δ^{10} (blue square). Figure 4.5 shows that the ratios of positional isomers are similar in sheep and cow adipose, however, in the cows' milk the *cis*-Δ^{11}:*cis*-Δ^{10} ratio is greater than the *trans*-Δ^{11}:*trans*-Δ^{10} ratio. Thus the *cis*-Δ^{11}/*trans*-Δ^{11} ratio appears to distinguish the reference cow adipose from reference sheep adipose, due to the higher abundance of the *trans*-Δ^{11} isomer in sheep adipose. It is yet to be seen, however, whether this ratio is significant in degraded animal fat residues since components with different stereochemistry may be affected by decay to differing extents.

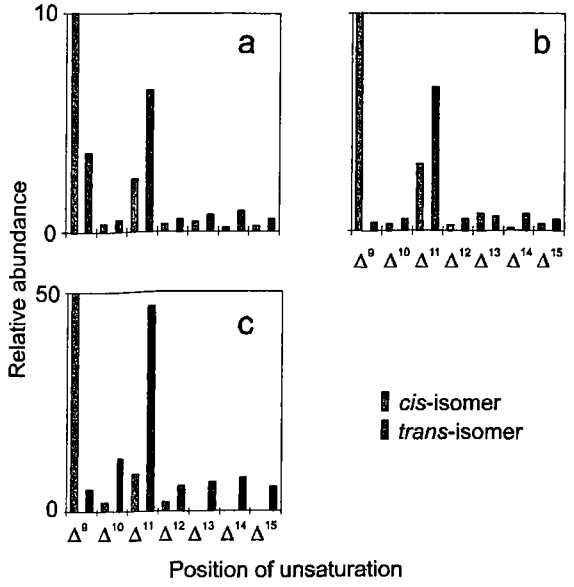


Figure 4.4 Distributions of positional and geometric isomers of $C_{18:1}$ in reference cow adipose fats: (a) C1BB and (b) C2BB, compared with bovine perinephric fat [(c); wgt %; Hay and Morrison, 1973].

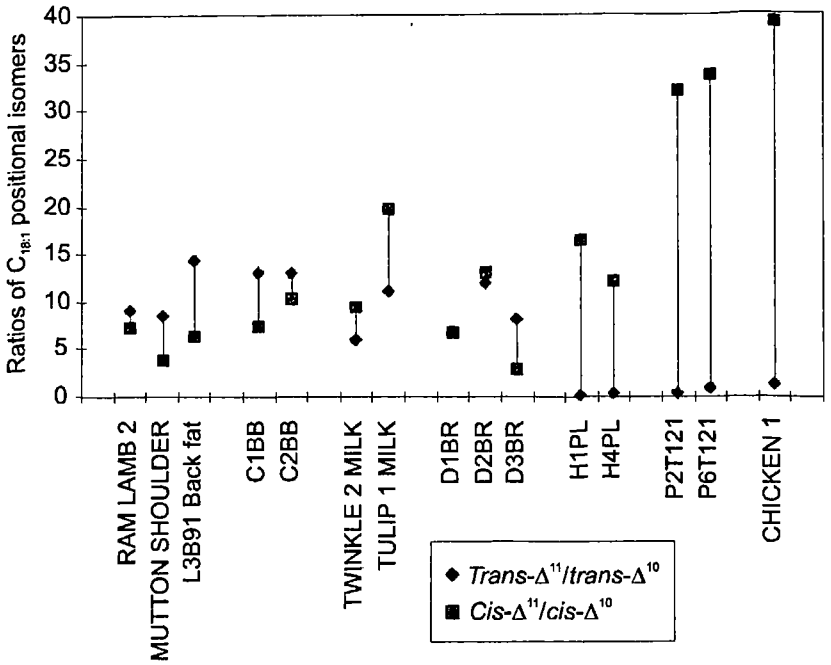


Figure 4.5 Relative abundances of the Δ^{10} and Δ^{11} *cis*- and *trans*-configured isomers in reference animal fats. Descriptions of the samples are given in Tables 2.1 to 2.9, Chapter 2.

4.2.2.2 Cow's milk

Cow's milk is similar in its proportion of C_{18} fatty acids to cow adipose fat, although it generally contains a greater proportion of $C_{14:0}$ and $C_{16:0}$ components. The ratio of mean $C_{16:0}$ and $C_{18:0}$ fatty acid abundances is 2.2:1, reflecting both the smaller proportion of $C_{18:0}$ and the increased $C_{16:0}$ component in cow's milk; the mean abundance of $C_{18:0}$ fatty acid is 16.5% in milk compared to 20.6% in adipose fat and for $C_{16:0}$ is 36.2% compared to 28.4% in milk and adipose fat, respectively. The important difference between milk and adipose fat from cattle is the presence, albeit not in very high abundance, of the shorter-chain ($C_{4:0}$ to $C_{14:0}$) fatty acids in milk fat. Milk fat also contains a higher abundance of $C_{14:0}$ (10.8%) compared with cow adipose which only comprises 3.8%. This is reflected in the higher ratio of $C_{14:0}/C_{17:0}$ fatty acids in the fats. The proportions of branched-chain and odd-carbon number fatty acids are similar in cow's adipose and milk fats (Tables 2 and 4, Appendix 3, pp. 359-360).

Some variation has been observed between the fatty acid composition of milk from different animals and of samples taken at different stages in the lactation cycle. The variation is clearly illustrated in Figure 4.3 in which the ratios of fatty acid components in

individual samples are compared. Milk from 'Sarah' prior to the birth of her calf comprised 47.0% $C_{16:0}$ and 11.2% $C_{18:0}$, whereas the sample of colostrum taken after the birth comprised 41.1% $C_{16:0}$ and 13.2% $C_{18:0}$. Similarly, milk samples taken from 'Mallard' pre- and 2 weeks post-calving comprised 35.8% and 14.8% compared with 23.9% and 35.6% $C_{16:0}$ and $C_{18:0}$ fatty acids, respectively, indicating that during the period when there is the greatest demand for milk, i.e. post calving, the proportion of $C_{18:0}$ in the milk increases at the expense of the $C_{16:0}$ fatty acid.

Cow's milk exhibits the same array of positional and geometric isomers as cow adipose fat, with the *cis*-Δ^9-isomer comprising 89% of the total $C_{18:1}$. As in cow adipose, the *trans*-Δ^{11} and *cis*-Δ^{11}-isomers are abundant relative to the other minor isomers and their ratio is quite distinctive (Fig. 4.6), however there is a higher abundance of the *cis*-Δ^{11}-isomer relative to the cow adipose fat.

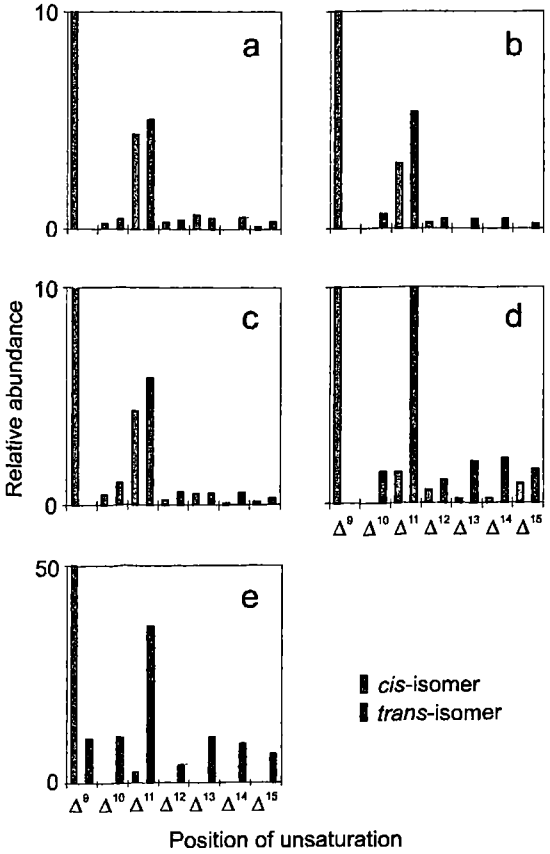


Figure 4.6 Distributions of positional and geometric isomers of $C_{18:1}$ in reference cow and sheep milk fats: (a) Tulip 1; (b) Sarah 1; (c) Twinkle 2; (d) Heb lamb 2, compared with butterfat [(e); wgt %; Hay and Morrison, 1970].

The relative abundances of the $C_{18:1}$ isomers in butterfat (from cow's milk) from Hay and Morrison (1970) are also shown in Figure 4.6. The scale is different in these histograms, since there is a significantly greater proportion of the *trans*-configured isomers in the Hay and Morrison (1970) data than in the dairy fats from our reference, C_3 pasture-reared animals. The *cis*-isomers are mainly absent in the study by Hay and Morrison (1970).

4.2.3 Ovine fats

4.2.3.1 Sheep adipose

The most noticeable difference between sheep and cow adipose is the greater proportion of $C_{18:0}$ in sheep adipose, which comprises a $C_{16:0}$ to $C_{18:0}$ fatty acid ratio of 0.7:1. Sheep adipose contains branched-chain and odd-carbon number fatty acids in similar abundance to cow adipose (Table 1, Appendix 3, p. 359). The $C_{17:0}$ fatty acid comprises 46% branched-chain and 54% aliphatic moieties. The compositions of fats from different animals were remarkably consistent considering the variation in age and breed of the different animals, and the fact that they were raised in different locations in the West Country. No correlation could be seen between age and fatty acid composition. The $C_{14:0}/C_{17:0}$ ratios observed in sheep adipose (mean=1.6) are significantly lower than in the sheep milk (mean=8.4), mirroring the data obtained for the cow adipose and milk fats.

The *cis*-Δ^9-isomer in sheep depot fat comprises a mean of 84% of the total $C_{18:1}$ and a greater relative abundance of the *trans*-Δ^{11}-isomer than is present in cow depot fat. The relative abundances of the minor components vary somewhat between different animals. The *trans*-isomers of Δ^{10} to Δ^{15} isomers are noticeably more abundant than their *cis*-counterparts. Figure 4.7 shows the distributions of isomers in sheep adipose. The ratios of *trans*-Δ^{11}:Δ^{10} and *cis*-Δ^{11}:Δ^{10} shown in Figure 4.5 vary between animals, however, the former ratio is always greater than the latter, as in the cow adipose fat.

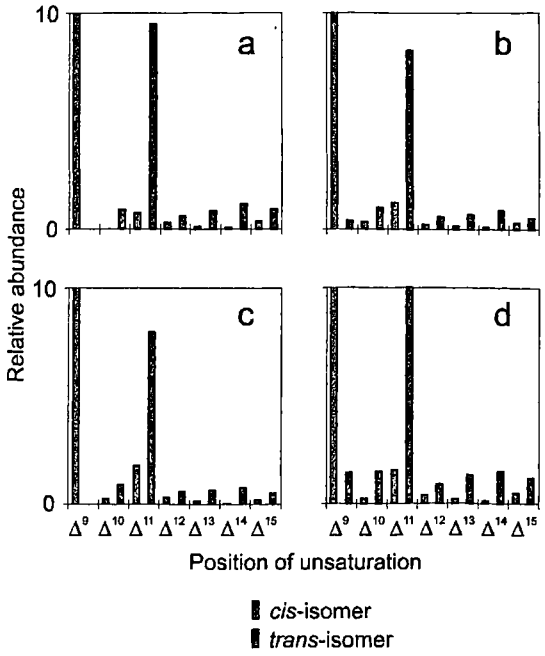


Figure 4.7 Distributions of positional and geometric isomers of $C_{18:1}$ in reference sheep adipose fats: (a) mutton leg fat (early '98); (b) mutton shoulder (early '98); (c) ram lamb 2, and (d) L3B91.

4.2.3.2 Sheep milk

Only two samples of sheep milk have been obtained to date from C_3-raised animals since the animals can only be milked whilst lambing. Sheep milk samples also contain the diagnostic shorter-chain fatty acids present in cow's milk (Fig. 4.2). The ratios of $C_{16:0}$ and $C_{18:0}$ fatty acids are 1:1 in the first sample and 1.2:1 in the second, thus both samples comprise similar proportions of the major saturated fatty acids. There is also a relatively high abundance of $C_{14:0}$ in sheep milk, which comprised 10.2%, compared with 2.9% in sheep adipose fat (Table 5, Appendix 3, p. 361).

In contrast to cow's milk fat, but consistent with sheep depot fat, the sheep milk comprises a greater abundance of the *trans*-Δ^{11}-isomer, thus giving a high *cis-trans* ratio of 10.9 compared with a mean of 1.4 in cow's milk. Due to the higher collective abundance of the Δ^{10} to Δ^{15} isomers in sheep milk, the major isomer (*cis*-Δ^9) comprised only 79% of the total. As in sheep adipose, the minor components are dominated by the *trans*-isomers rather than the *cis*-isomers, with a greater abundance of the *trans*-Δ^{13} and Δ^{14} compared with the trans-Δ^{12} and Δ^{15}.

4.2.4 Cervine fats

The greatest variation in fat composition between individual animals has been seen in the adipose fat of deer, although overall compositions are not dissimilar to other ruminant adipose fats, with the predominance of $C_{16:0}$, $C_{18:0}$ and $C_{18:1}$ components and minor $C_{18:2}$ and $C_{18:3}$ (Table 3, Appendix 3, p. 360). The branched-chain and aliphatic C_{15} and C_{17} components are present, with equal abundances of the C_{17} components. There are similar proportions of the saturated C_{16} and C_{18} fatty acids, in the ratio 0.9:1.

The distribution of $C_{18:1}$ positional isomers is similar to that of the other ruminant fats with the *cis*-Δ^9-isomer predominant (76%). There is some inconsistency between the relative abundances of $C_{18:1}$ isomers in samples from different animals, however, some similarity is seen in the dominance of the *trans*-isomers amongst the minor components and the relatively high abundance of *cis*- and *trans*-Δ^{11} components (Fig. 4.8). The abundance of the *trans*-Δ^{10} is two-fold higher than in cow adipose and there is a higher *cis*-Δ^{11}:*trans*-Δ^{11} ratio than in sheep adipose. Figure 4.5 shows two of the deer fat samples have comparable *trans*-Δ^{11}:Δ^{10} and *cis*-Δ^{11}:Δ^{10} ratios, however sample D3BR differs due to the relatively high abundance of the *cis*-Δ^{10} fatty acid.

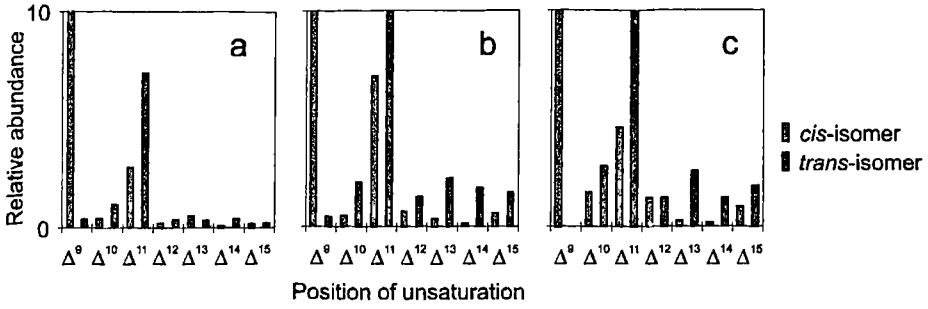


Figure 4.8 Distributions of positional and geometric isomers of $C_{18:1}$ in reference deer adipose fats: (a) D1BR; (b) D2BR, and (c) D3BR.

4.2.5 Equine fats

The most obvious differences between the fats from ruminant (Fig. 4.2) and non-ruminant (Fig. 4.9) animals is the presence of a greater abundance of the di- and trienoic C_{18} components and the lower proportions of odd-carbon number and branched-chain fatty acids in pseudo-ruminant (e.g. equines) and other non-ruminant fats. There is a greater proportion of unsaturation in horse fat than in true ruminants at the expense of the saturated C_{18} fatty acid, with greater than 60% of the total fatty acid in horse fat

comprising of unsaturated C_{16} and C_{18} components (Table 7, Appendix 3, p. 362). The ratio of C_{16} and C_{18} fatty acids in horse fat is 4:1 (Fig. 4.10). The $C_{15:0}$ and $C_{17:0}$ straight-chain fatty acids and $C_{17:0}$ branched-chain fatty acids are present only in trace amounts (<0.7%), with the straight-chain C_{17} components comprising 75% of the total C_{17} fatty acids.

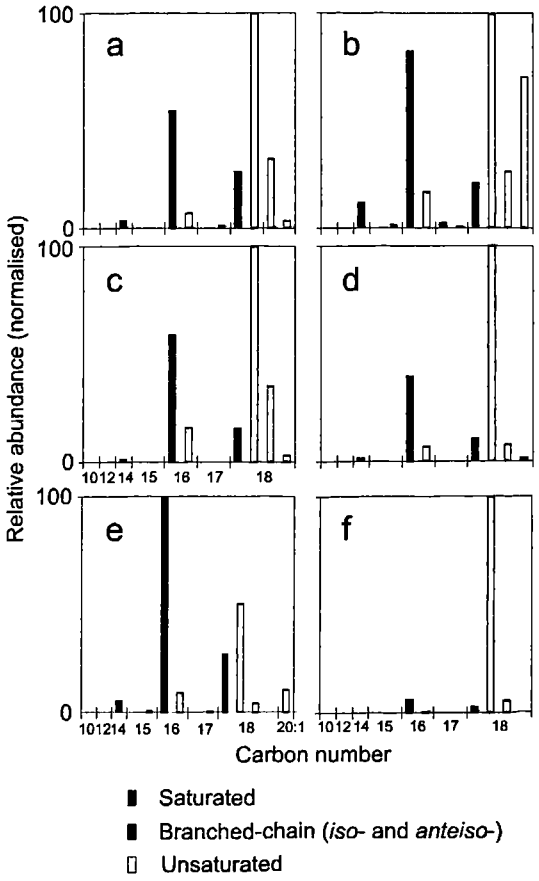


Figure 4.9 Relative abundances of fatty acids in reference non-ruminant fats and oils: (a) pig adipose (n=9); (b) horse adipose (n=10); (c) chicken fat (n=3); (d) goose fat (n=4); (e) fish oil (n=3), and (f) virgin olive oil (n=1).

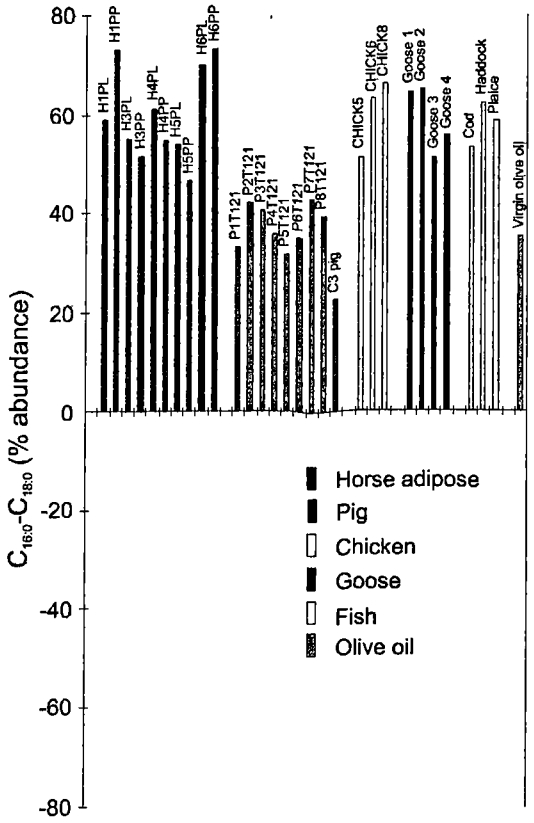


Figure 4.10 Relative abundances of $C_{16:0}$ and $C_{18:0}$ fatty acids in reference non-ruminant fats and oils.

The distribution of $C_{18:1}$ isomers in the reference horse fat is also distinctly different than that of ruminant fats, with fewer isomers present and in lower abundance (Fig. 4.11). The *cis*-Δ^9 isomer constitutes 93% (mean value) of the total $C_{18:1}$ fatty acids. The major difference between these and other fats described thus far is the dominance of the *cis*-Δ^{11}-isomer over all the other minor components which collectively constitute less than 1.5% (mean) of the total $C_{18:1}$. Significantly, the *trans*-Δ^{11}-isomer is minor or absent from the reference horse depot fats. Figure 4.5 shows that the horse adipose fats can be distinguished from the cow and sheep adipose fats using the difference between the two ratios shown, and furthermore, that the *trans*-Δ^{11}:Δ^{10} ratio is quite distinct from all of the ruminant fats because of the relatively low abundance of the *trans*-Δ^{11} fatty acid.

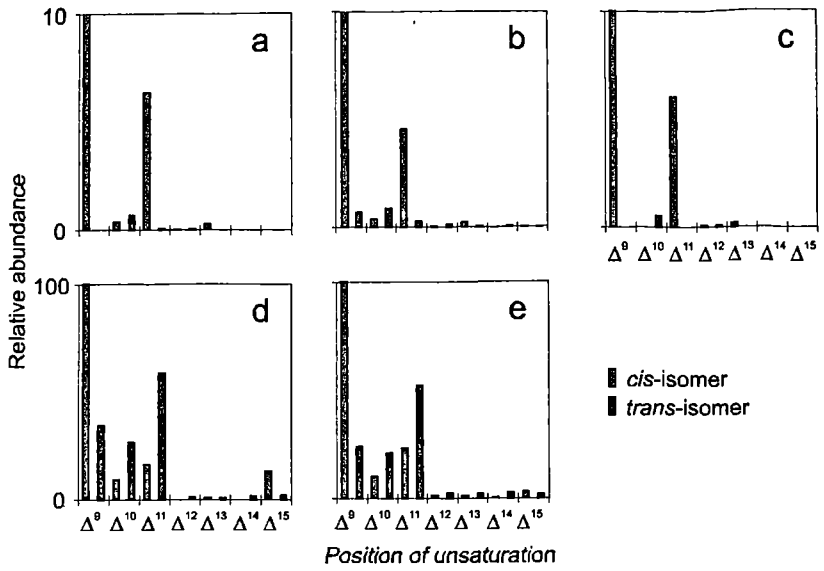


Figure 4.11 Distributions of positional and geometric isomers of $C_{18:1}$ in reference horse adipose fats: (a) H1PL; (b) H4PL; (c) H5PP, compared with (d) subcutaneous horse fat (skin, H1) and (e) internal horse fat (sacrum, H1) from the Siberian permafrost burial.

4.2.6 Porcine fats

Porcine adipose is also highly unsaturated, comprising >60% unsaturated C_{16} and C_{18} components (Table 6, Appendix 3, p. 361). The ratio of abundance of $C_{16:0}$ to $C_{18:0}$ fatty acids is 2.1:1. Branched-chain fatty acids were undetectable in the porcine subcutaneous adipose fats, and C_{17} aliphatic fatty acids comprise only 0.4%. Furthermore, there are no short-chain ($<C_{14}$) fatty acids and $C_{14:0}$ comprises only 1.3% of the total.

Porcine fats contain fewer positional isomers of $C_{18:1}$ than ruminant fats, and they are present in significantly lower abundance (Fig. 4.12). The *cis*-Δ^9 fatty acid comprises 90% of the total and there are only minor amounts of *trans*-fatty acids, constituting <0.7%. The *cis*-Δ^{11}-isomer dominates the minor components, as in reference horse fat. The *trans*-Δ^{11}:Δ^{10} and *cis*-Δ^{11}:Δ^{10} ratios shown in Figure 4.5 enable distinctions to be drawn between the porcine fats and ruminant fats. The characteristics of the $C_{18:1}$ isomers in the porcine fats are not dissimilar to those seen in the horse fats, however, the porcine fats have a much higher proportion of *cis*-Δ^{11} fatty acids.

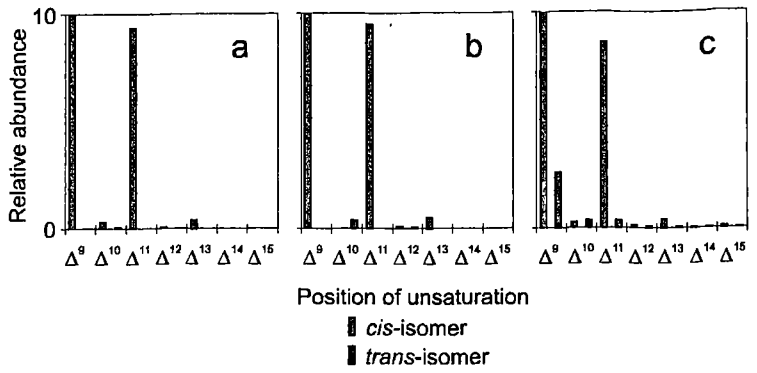


Figure 4.12 Distributions of positional and geometric isomers of $C_{18:1}$ in reference porcine adipose fats: (a) P2T121; (b) P3T121, and (c) P6T121.

4.2.7 Poultry fats

4.2.7.1 Chicken

The poultry fats are also highly unsaturated compared with ruminant depot fats, and chicken is very similar in composition to porcine adipose fat, except for a lower abundance of $C_{14:0}$ and a slightly higher abundance of $C_{16:1}$ (Table 8, Appendix 3, p. 362). The mean ratio of $C_{16:0}$:$C_{18:0}$ fatty acids is 3.9:1. No odd-carbon number or branched-chain fatty acids were detected in the chicken fats.

The $C_{18:1}$ components in chicken depot fats are similar to porcine fats with the *cis*-Δ^9-isomer predominant (Fig. 4.13). The *cis*-Δ^{11} is the only other component present in significant abundance. The similarity between chicken and porcine fats and the distinction between these and other fats based on the distributions of $C_{18:1}$ fatty acids is illustrated in Figure 4.5.

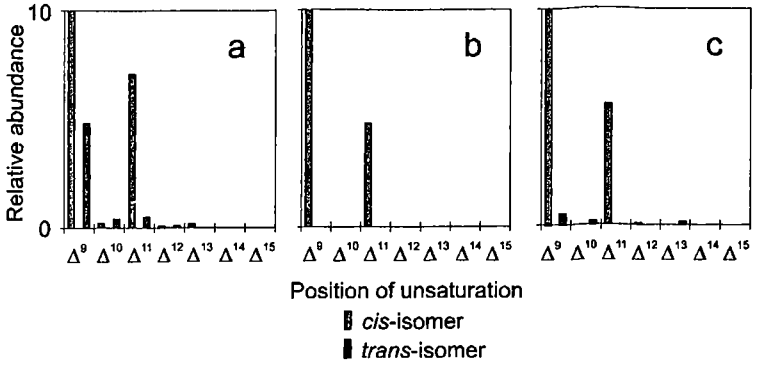


Figure 4.13 Distributions of positional and geometric isomers of $C_{18:1}$ in reference chicken fats: (a) Chicken 1; (b) Chicken 2, and (c) Chicken 5.

4.2.7.2 Goose

Goose fat comprises 58-62% $C_{18:1}$ and 21-28% $C_{16:0}$ fatty acids, with minor amounts of $C_{16:1}$, $C_{18:0}$ and $C_{18:1}$, with a mean $C_{16:0}$:$C_{18:0}$ ratio of 3.9:1 (Table 9, Appendix 3, p. 362). No odd-carbon number or branched-chain fatty acids were detected in goose fat. Similar to the chicken and porcine fats, goose fat comprises predominantly *cis*-Δ^9, with relatively minor amounts of *cis*-Δ^{11} (Fig. 4.14) and the absence of the *trans*-Δ^{11} component.

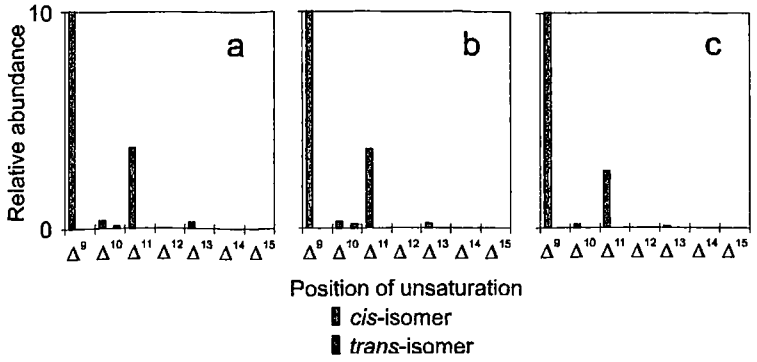


Figure 4.14 Distributions of positional and geometric isomers of $C_{18:1}$ in reference goose fats: (a) Goose 1; (b) Goose 2, and (c) Goose 3.

4.2.8 Fish oils

Examples of three salt-water fish have been analysed for comparison, although data from the literature should also be suitable for comparison. As in poultry and non-ruminant fats there is a high abundance of $C_{16:0}$ compared with $C_{18:0}$ fatty acid: 3.3:1 for cod, 4.3:1 for haddock and 3.9:1 for plaice, but a lesser proportion of unsaturated fatty acids (Table 10, Appendix 3, p. 362). There are no fatty acids with <14 carbons and none of the minor branched-chain or odd-carbon number components common in ruminant animal fats, although other workers have detected minor abundances of short-chain fatty acids in fish oils. $C_{20:1}$ comprised a mean of 5.1% in the fish analysed. The distributions of positional isomers of $C_{18:1}$ in fish tissues were not investigated in detail.

4.2.9 Olive oil

The composition of virgin olive oil was investigated for comparison with reference animal fats and fish oils. It is well-known that the major component in olive oil is the $C_{18:1}$ component, which in the sample analysed comprised 87% of the total fatty acids (Table 11, Appendix 3, p. 363). The di- and trienoic C_{18} components comprised 4.7% and 0.2%,

respectively. Saturated C_{16} and C_{18} fatty acids made up only 7.5% of the total in a ratio of 2.1:1.

Positional isomer analysis has shown that the *cis*-Δ^9 component, of which olive oil is known to comprise a high proportion, actually constituted 97.9% of the $C_{18:1}$ fatty acid in the sample analysed. The remaining 2.1% comprised the *cis*-Δ^{11}-isomer.

4.2.10 Mixtures of different fats

As has been seen in Section 4.2.3.1, one of the most promising uses of the $C_{16:0}$ and $C_{18:0}$ fatty acid ratios is in distinguishing between sheep and cow adipose fats. Since sheep and cows are thought to have been among the most common domesticated species in antiquity, the proportions of $C_{16:0}$ and $C_{18:0}$ fatty acids have been calculated since they may reflect different contributions of these two fats present in a mixture. Table 4.1 indicates the results of a calculation based on the theoretical mixing of different proportions of cow and sheep adipose fats (by weight).

Table 4.1 Ratios of fatty acids present in mixtures of adipose fats (based on mean values obtained for fatty acid abundances in reference fats).

Proportions of fats present	**% abundance of fatty acid**	
	$C_{16:0}$	**$C_{18:0}$**
100% sheep	43.0	57.0
75% sheep, 25% cow	46.7	53.3
50% sheep, 50% cow	50.5	49.5
25% sheep, 75% cow	54.2	45.8
100% cow	57.9	42.1

Additionally, mixtures of different reference fats were prepared by weight and the distributions of positional isomers in the mixtures analysed in order to observe their distributions in mixtures of different proportions of fats. The results of mixing porcine and cow adipose fats are shown in Figure 4.15 (data are shown in Table 2, Appendix 4, p. 375). The most obvious change between 100% porcine and 100% cow fat is the increased contribution of the *trans*-Δ^{11} isomer and an overall increase in the abundance of the minor *cis*- and *trans*-isomers across the whole range of positions. Similarly, this trend is reflected in Figure 4.16 where porcine adipose fat is mixed with lamb adipose fat. Figure 4.17(a)

and (b) illustrate the effect of mixing fats on the ratios of $C_{18:1}$ fatty acids. The major differences seen between 100% ruminant and 100% porcine fats are caused by the higher proportion of the *cis*-Δ^{11} component in the porcine fats.

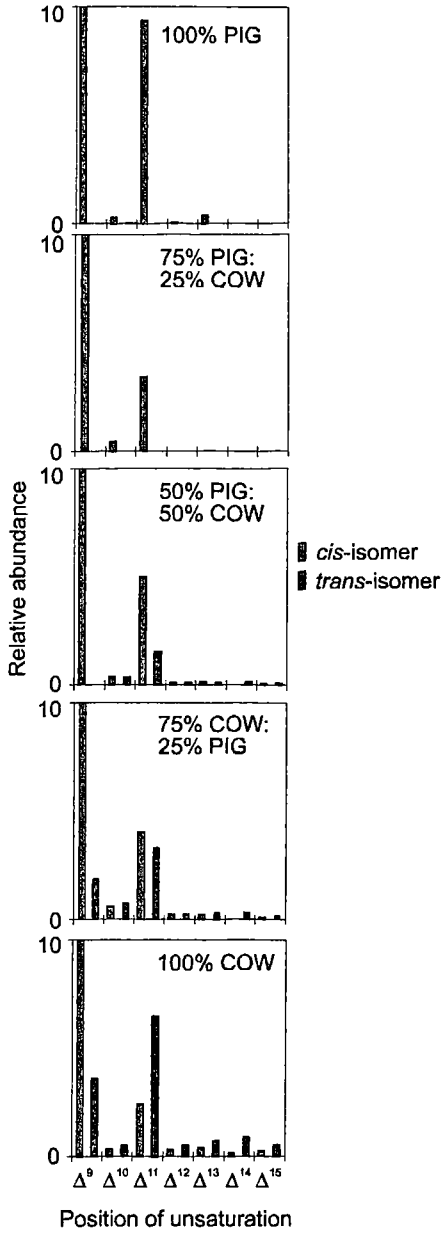


Figure 4.15 Distributions of positional and geometric isomers of $C_{18:1}$ in mixtures of reference cow and pig adipose fats.

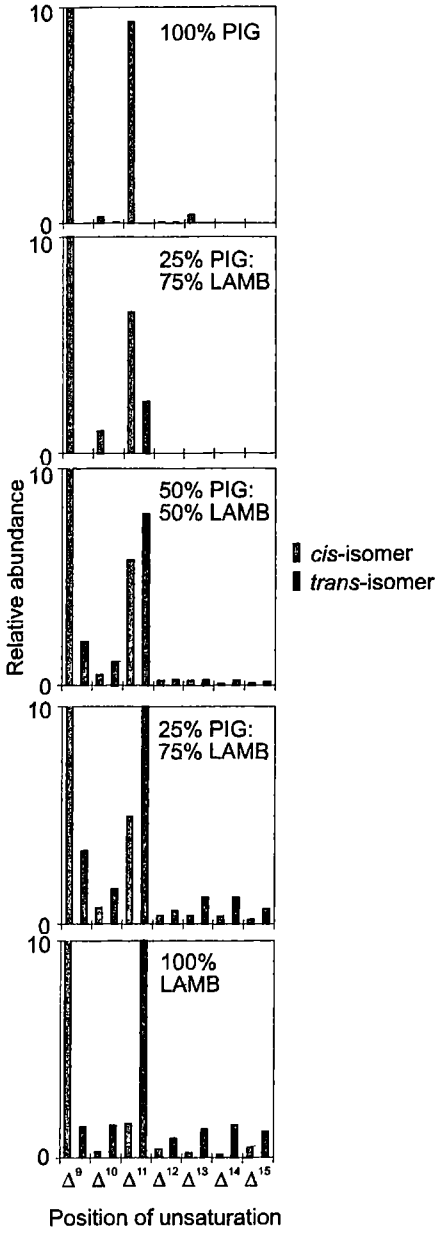


Figure 4.16 Distributions of positional and geometric isomers of $C_{18:1}$ in mixtures of reference lamb and pig adipose fats.

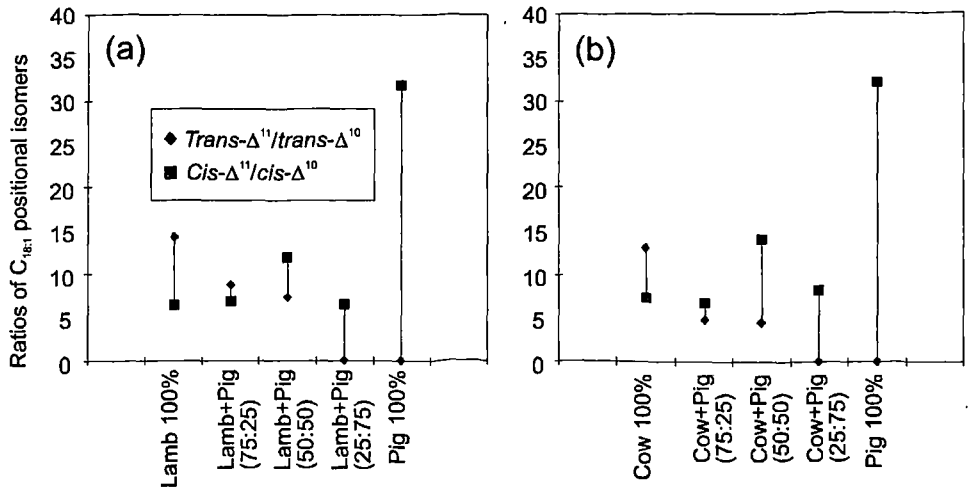


Figure 4.17 Relative abundances of the $C_{18:1}\Delta^{10}$ and Δ^{11} *cis*- and *trans*-configured isomers in mixtures of reference animal fats: (a) lamb and pig, and (b) cow and pig.

4.3 Analysis of fatty acid distributions in archaeological and ethnographic residues

The overall lipid distributions in extracts from the archaeological and ethnographic vessels have been discussed briefly in Chapter 3; this Section now considers the distributions of fatty acid components in greater detail. Solvent extracts of the archaeological potsherds and ethnographic vessels were saponified and converted to their methyl ester derivatives as described in Sections 9.1.5 and 9.1.6, and submitted to analysis by GC (Section 9.2.1). Where $C_{18:1}$ fatty acids had been preserved in pottery residues, analysis of the positional and geometric isomers was carried out following preparation of their DMDS derivatives (Section 9.1.7) and analysis by GC/MS (Section 9.2.2).

4.3.1 Sites with well-documented faunal assemblages

4.3.1.1 West Cotton (Late Saxon/early medieval)

Figure 4.18 shows the difference in % abundance between the $C_{16:0}$ and $C_{18:0}$ fatty acids in extracts from the West Cotton sherds. Clearly, the majority (76%) of the extracts comprise a greater proportion of the $C_{18:0}$ fatty acid than the $C_{16:0}$ component which indicates that these samples may derive from sheep adipose fats, while 4 extracts (14%) comprise similar proportions of the two major fatty acids and only 3 (10%) comprise a greater abundance of the $C_{16:0}$ fatty acid. The relative abundances of saturated free fatty acids, including $C_{14:0}$, $C_{16:0}$, $C_{17:0}$, $C_{18:0}$ and branched-chain $C_{17:0}$ are given in Table 12, Appendix 3 (p. 363). All of the extracts from West Cotton comprise the range of even and odd carbon-number fatty

acids, with the exception of RP4, 10 and 88, in which there are no branched-chain $C_{17:0}$ components. The $C_{14:0}/C_{17:0}$ ratios for the West Cotton extracts are given in Table 24, Appendix 3 (pp. 371-373), and provide an indication of the relative abundance of the $C_{14:0}$ component in the different samples since the abundance of the $C_{17:0}$ fatty acid is more consistently present in similar proportions than the $C_{14:0}$. Four West Cotton vessels have $C_{14:0}/C_{17:0}$ ratios above 3.0, namely RP91, 22, 30 and WC30. Extracts with a ratio between 2.0 and 3.0 have come from vessels RP2, 73, 60, 72, 16 and 93. The higher ratios may be indicative of dairy fats since the same ratio obtained for the reference milk fats ranges between 3.1 and 15.4 with a mean of 9.6, which is higher than the mean ratio in the reference adipose fats.

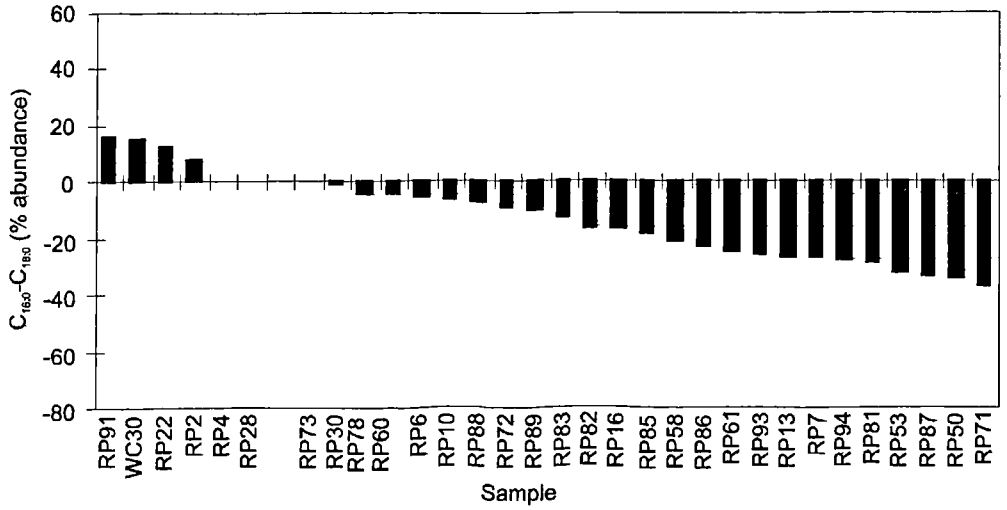


Figure 4.18 Relative abundances of $C_{16:0}$ and $C_{18:0}$ fatty acids in remnant fats from the Late Saxon/early medieval West Cotton assemblage. Descriptions of the samples are given in Table 1, Appendix 1 (pp. 333-337).

Positional and geometric isomer distributions were measured for 21 of the West Cotton extracts (Figs. 4.19 and 4.20; Table 5, Appendix 4, p. 376). The *trans*-configured positional isomers are the most abundant in almost all cases with the *cis*-isomers predominantly in the Δ^9 position. RP2 and RP4 contain relatively low abundances of Δ^{10} to Δ^{15} positional isomers relative to the other extracts. RP6, 82 and 89 appear to comprise a greater predominance of the *trans*-Δ^{10}, Δ^{12}, Δ^{13}, Δ^{14} and Δ^{15} isomers, however this is probably misleading and is actually reflecting the loss of the *cis*-Δ^9 which is predominant in fresh fats.

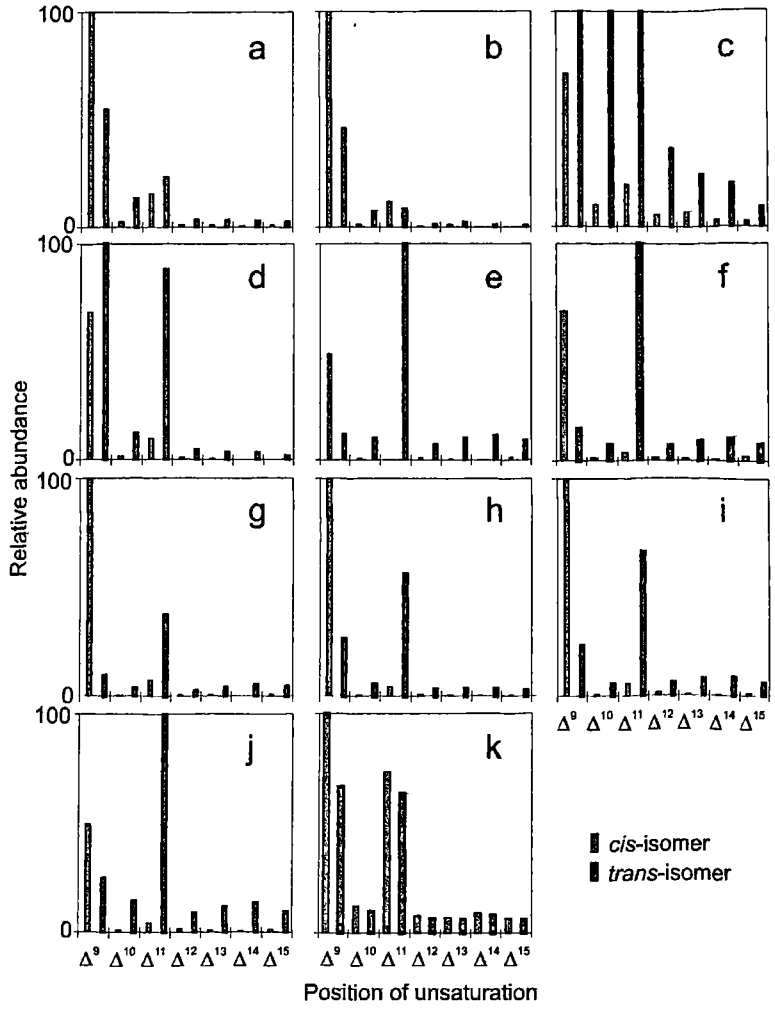


Figure 4.19 Distributions of positional and geometric isomers of $C_{18:1}$ in remnant fats from the Late Saxon/early medieval West Cotton assemblage: (a) RP2; (b) RP4; (c) RP6; (d) RP7; (e) RP16; (f) RP30; (g) RP50; (h) RP53; (i) RP58; (j) RP60, and (k) WC30.

In the cases of RP16, 30, 50, 53, 58, 60, 71, 72, 78, 83, 86, 87, 91, 93 and 94, a similar pattern of positional and geometric $C_{18:1}$ isomers occurs, with all the *trans*-configured isomers present and the *trans*-Δ^{11} present in relatively high abundance. In extracts from RP2, 4, 6, 82, and 89 the *trans*-Δ^{11} isomer is no more abundant than the other *trans*-isomers. The *cis*-isomers are present in varying abundance which may be influenced by the extent of decay. The relative abundances of the *trans*-isomers would be expected to be more diagnostic. Since all the reference fats and oils analysed have shown a predominance of either the *cis*- or the *trans*-form of the Δ^{11} component, the absence of both geometric isomers as seen in some of the archaeological fats indicates that the *cis*-isomer may have originally been present as the dominant component in the intact fat, but has been lost due

to its preferential decay. Extracts from RP2 and RP4 comprise comparable distributions of $C_{18:1}$ isomers and the distributions of $C_{16:0}$ and $C_{18:0}$ fatty acids are also similar, both containing a greater abundance of the $C_{16:0}$ fatty acid. However in comparison, RP91 which also comprises a greater proportion of the $C_{16:0}$ fatty acid has a very different distribution of $C_{18:1}$ isomers from RP2 and RP4.

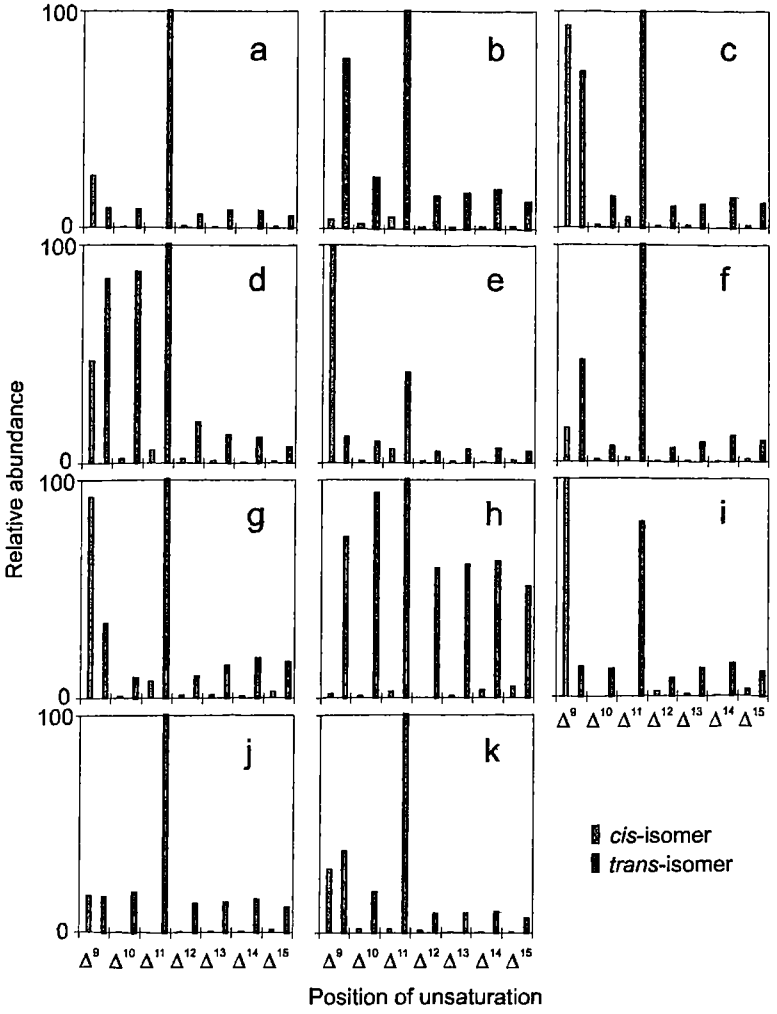


Figure 4.20 Distributions of positional and geometric isomers of $C_{18:1}$ in remnant fats from the Late Saxon/early medieval West Cotton assemblage: (a) RP71; (b) RP72; (c) RP78; (d) RP82; (e) RP83; (f) RP86; (g) RP87; (h) RP89; (i) RP91; (j) RP93, and (k) RP94.

Figure 4.21 shows the *trans*-Δ^{11}:Δ^{10} and *cis*-Δ^{11}:Δ^{10} ratios, in which samples RP2 and RP4 show ratios comparable to the non-ruminant reference fats and the cows milk reference fats. RP30, 60, 78, 72, 58, 86, 94, 53 have a lower *cis*-Δ^{11}:Δ^{10} ratio than *trans*-Δ^{11}:Δ^{10} ratio, and RP16, 93 and 71 do not contain any of the *cis*-Δ^{11} fatty acid. RP 91 contains no *cis*-

configured isomers. RP89, 83, 82, 87 and 50 have a lower *trans*-Δ^{11}:Δ^{10} ratio than *cis*-Δ^{11}:Δ^{10} ratio.

WC30, discussed in Section 1.4, and shown in Figure 1.4 has been reanalysed as part of this research project due to its complex lipid profile. The proportion of $C_{16:0}$ fatty acid is higher than the $C_{18:0}$ fatty acid and thus in Figure 4.21 the ratio is comparable with RP91. WC30 also comprises straight- and branched-chain saturated fatty acids indicative of a ruminant fat, however the distribution of positional isomers of the $C_{18:1}$ fatty acid is significantly different from the other samples from West Cotton. In this case the *cis*-configured isomers appear better preserved. The *cis*- and *trans*-Δ^9 and Δ^{11} are both present in abundance. The distribution of *trans*-configured isomers (all that remain) in RP91 compare well with those in WC30, with the exception of the *trans*-Δ^9 isomer which is present in higher abundance in WC30.

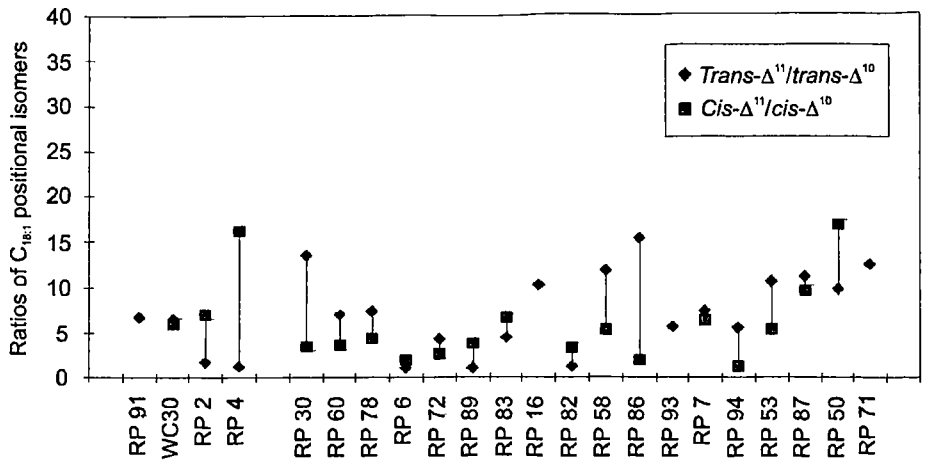


Figure 4.21 Relative abundances of the $C_{18:1}$ Δ^{10} and Δ^{11} *cis*- and *trans*-configured isomers in remnant fats from the West Cotton assemblage.

4.3.1.2 Stanwick (Iron Age/Romano-British)

The ratios of abundance of $C_{16:0}$ and $C_{18:0}$ fatty acids in Stanwick extracts is shown are Figure 4.22. The data are comparable with that seen in West Cotton extracts, with 79% of extracts comprising a greater proportion of $C_{18:0}$ than $C_{16:0}$ and only 16% comprising a lesser proportion of the $C_{18:0}$. Only one extract, namely ST197, contains $C_{16:0}$ and $C_{18:0}$ fatty acids in equal abundance. All of the extracts from Stanwick comprise saturated $C_{14:0}$, $C_{16:0}$, straight- and branched-chain $C_{17:0}$ and $C_{18:0}$ fatty acids for which the data are

presented in Table 13, Appendix 3 (p. 364). The higher $C_{14:0}/C_{17:0}$ fatty acid ratios (Table 24, Appendix 3, pp. 371-373) correspond to the samples containing a higher abundance of $C_{16:0}$ than $C_{18:0}$ fatty acid, whereas the lower ratios (<1.0) correspond to the samples with less $C_{16:0}$ than $C_{18:0}$ fatty acid. Thus the $C_{17:0}$ fatty acid is in greater abundance than the $C_{14:0}$ fatty acid in the latter samples. These data may be useful in identifying dairy fats, since the reference milk fats comprised higher mean $C_{14:0}/C_{17:0}$ ratios than the reference ruminant adipose fats.

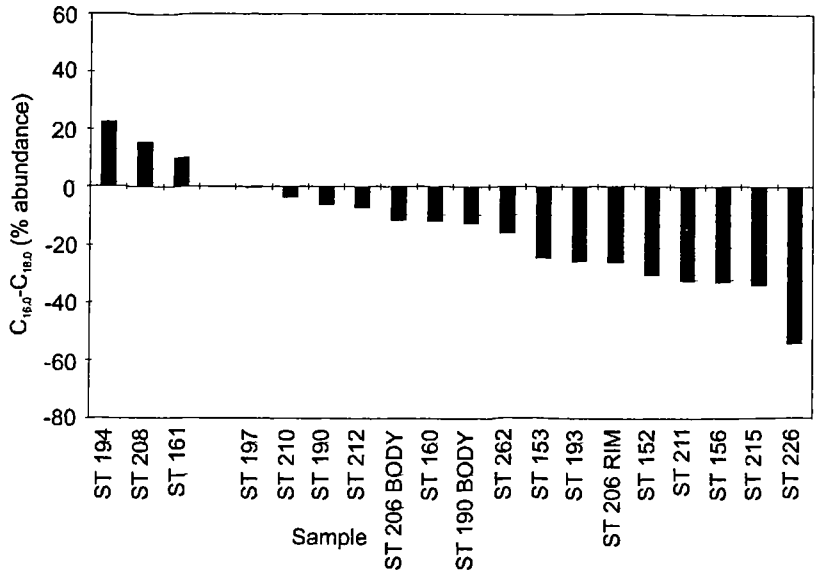


Figure 4.22 Relative abundances of $C_{16:0}$ and $C_{18:0}$ fatty acids in remnant fats from the Iron Age/Romano-British Stanwick assemblage. Descriptions of the samples are given in Table 2, Appendix 1 (pp. 338-339).

Six of the Stanwick extracts were analysed to determine positional isomers of the $C_{18:1}$ fatty acid. Data are shown in Figure 4.23 (Table 6, Appendix 4, p. 377). Preservation of the $C_{18:1}$ components is comparable to that seen in the majority of the West Cotton extracts, indicated by the similar proportion of *cis*-configured isomers present. The distributions of isomers are similar to the distributions seen in the majority of the extracts from West Cotton. With the exception of ST211, the Δ^{11} dominates the *trans*-configured isomers; in ST211 the *trans*-Δ^9 is in greater abundance. ST212 is dominated by the high proportion of *cis*-Δ^9 present. Although ST194 differs from the other extracts analysed on the basis of the ratio of $C_{16:0}$ to $C_{18:0}$ fatty acids present, the distribution of isomers of the $C_{18:1}$ fatty acid is not dissimilar to the other samples. ST211, which comprised a much higher abundance of

the $C_{18:0}$ compared to the $C_{16:0}$ fatty acid, also has a higher relative abundance of the $C_{18:1}$ *trans*-Δ^{10} and *cis*-Δ^{11} components.

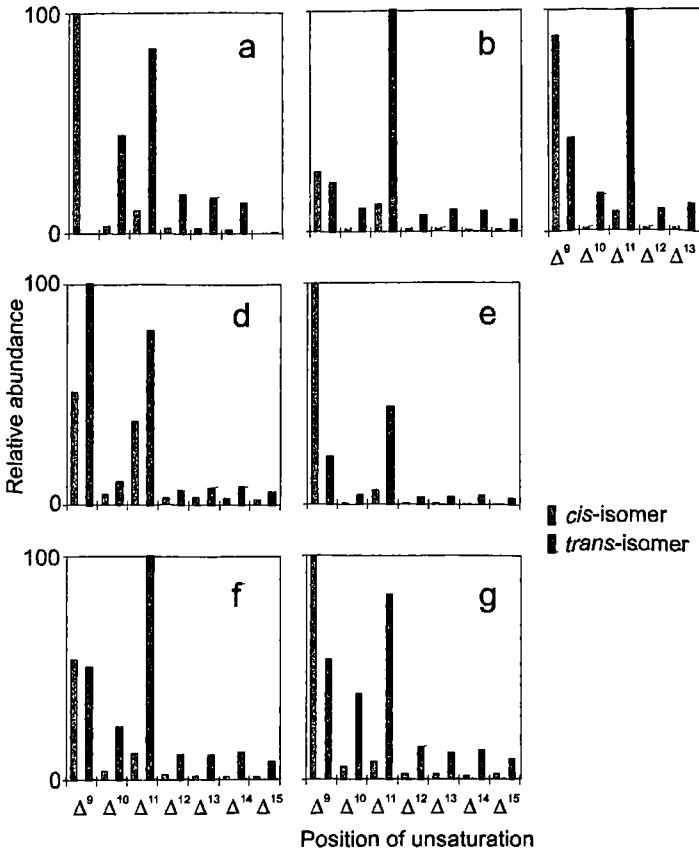


Figure 4.23 Distributions of positional and geometric isomers of $C_{18:1}$ in remnant fats from the Stanwick assemblage: (a) ST190; (b) ST194; (c) ST210; (d) ST211; (e) ST212; (f) ST206 BODY, and (g) ST206 RIM.

Figure 4.24 shows that the ratios of the *trans*-Δ^{11}:Δ^{10} and *cis*-Δ^{11}:Δ^{10} are the same in samples ST94, 210 and 211. In samples ST212, 206 body and 206 rim the *cis*-Δ^{11}:Δ^{10} ratio is slightly lower than the *trans*-Δ^{11}:Δ^{10} ratio and the opposite is the case in sample ST190. None of the samples from Stanwick exhibit the distinct characteristics corresponding to the reference non-ruminant adipose fats.

Extracts from ST206 body and ST206 rim sherds comprise similar fatty acid profiles, with a greater abundance of the $C_{18:0}$ fatty acid over the $C_{16:0}$, and, in addition, the distributions of $C_{18:1}$ positional isomers are comparable (Fig. 4.23). The similarity between the two remnant fats from different sherds of the same vessel is indicative that the distributions which we are seeing actually reflect the characteristics of the fat and are not distorted by

contamination during burial nor subject to change during sample preparation. The only difference is the extent to which the *cis*-Δ^9 isomer has decayed, since the abundance of this component is slightly higher in ST206 rim.

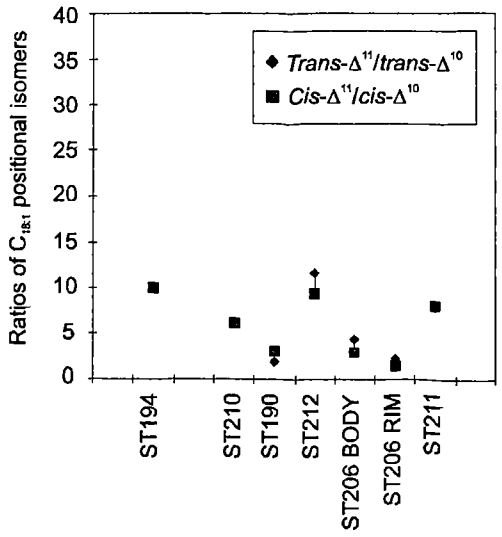


Figure 4.24 Relative abundances of the $C_{18:1}$ Δ^{10} and Δ^{11} *cis*- and *trans*-configured isomers in remnant fats from the Stanwick assemblage.

4.3.2 Sites with an unusually strong bias in the faunal record

4.3.2.1 Wickham Bonhunt (Romano-British/Middle Saxon)

The distributions of $C_{16:0}$ and $C_{18:0}$ fatty acids in 12 extracts from Wicken Bonhunt sherds are shown in Figure 4.25. With the exception of sample no. 16, the $C_{16:0}$ fatty acids were present in lower abundance than the $C_{18:0}$ fatty acid. This $C_{16:0}$:$C_{18:0}$ ratio is particularly low in samples 3, 12, 13 and 19. Sample nos. 3, 8 and 16 comprised noticeably lower abundances of the $C_{17:0}$ straight- and branched-chain fatty acids than the other extracts (see Table 14, Appendix 3, p. 364). The ratio of $C_{14:0}/C_{17:0}$ fatty acids is lower than 2.0 in all samples from Wicken Bonhunt, and in three samples (nos. 1, 12 and 13) the abundance of the $C_{17:0}$ fatty acid is greater than the $C_{14:0}$ (Table 24, Appendix 3, pp. 371-373).

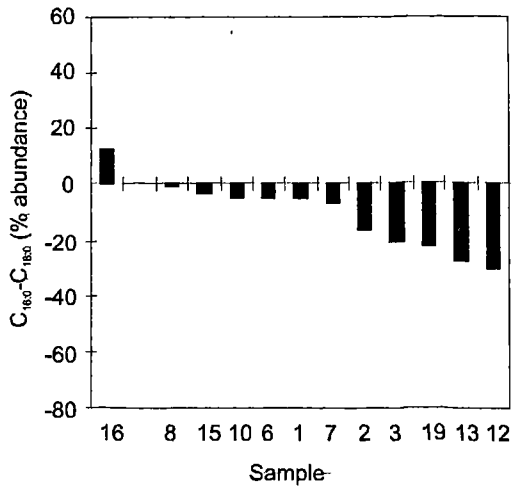


Figure 4.25 Relative abundances of $C_{16:0}$ and $C_{18:0}$ fatty acids in remnant fats from the Late Saxon Wicken Bonhunt assemblage. Descriptions of the samples are given in Table 3, Appendix 1 (p. 340).

The distributions of $C_{18:1}$ positional and geometric isomers of 8 of the Wicken Bonhunt extracts were determined and are shown in Figure 4.26 (Table 7, Appendix 4, p. 377). The *cis*-configured isomers are notably more abundant in extracts from these Saxon sherds than in those from Stanwick (Iron Age/Romano-British) or West Cotton (Late Saxon/early medieval) sherds. In all of the extracts, the *cis*-Δ^9 isomers comprise greater than 63% of the total $C_{18:1}$ fatty acid, indicating that decay has been minimal and that the components present still closely reflect the original distributions in the fats. In sample no. 3, the *cis*-Δ^9 isomer comprises 91.6% of the total $C_{18:1}$. In samples 3, 8, 12, 15 and 16, there is a relatively high abundance of the *cis*-Δ^{11} component, such as has only previously been seen in samples RP2, RP4 and WC30 from West Cotton and ST211 from Stanwick. Sample nos. 2 and 7 comprise a relatively high abundance of *trans*-Δ^{11}, and although this is also true of sample no 10, this may be due to the loss through degradation of *cis*-configured isomers from the latter sample. A useful measure of the extent of decay of the *cis*-isomers may be taken from the presence or absence of *cis*-configured isomers in positions $\Delta^{13,\ 14}$ and Δ^{15} since positional isomers in fresh fats generally exist in both *cis*- and *trans*-forms, and additionally from the % abundance of the *cis*-Δ^9 isomer present. All of the samples from Wicken Bonhunt comprise greater *cis*-Δ^{11}:Δ^{10} ratios than *trans*-Δ^{11}:Δ^{10} ratios. The ratios for samples WKB16, 8 and 15 shown in Figure 4.27 compare well with the ratios in the reference non-ruminant fats due to the low abundance of the *trans*-Δ^{11} component.

Samples WKB2 and 3 do not contain the *cis*-Δ^{10} isomer and thus also exhibit a high *cis*-Δ^{11}:Δ^{10} ratio and are comparable with the reference non-ruminant fat. WKB12 comprises a greater abundance of the *cis*-Δ^{10}-isomer than the other extracts from this site.

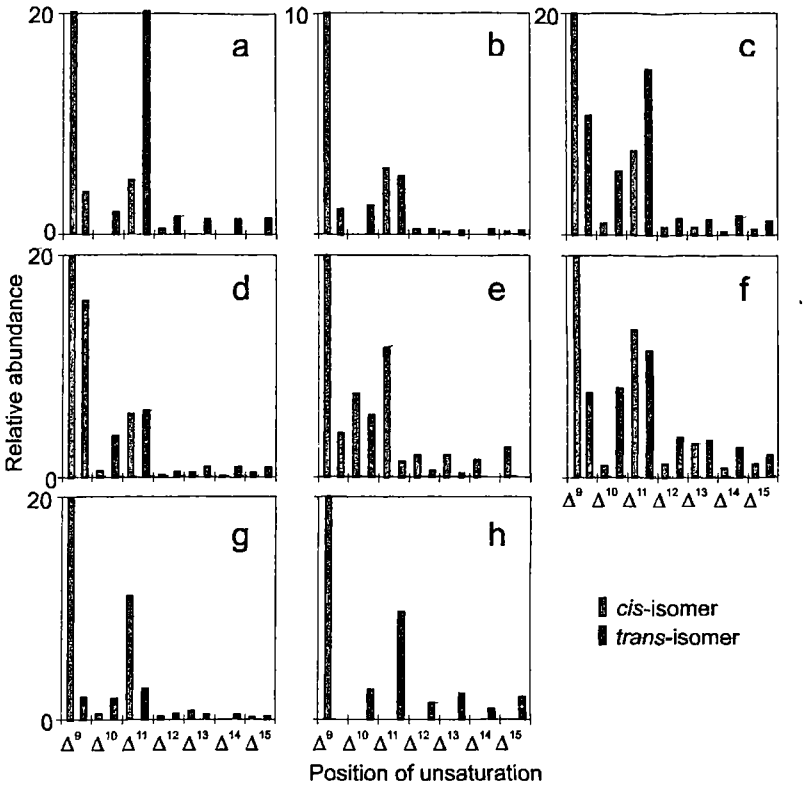


Figure 4.26 Distributions of positional and geometric isomers of $C_{18:1}$ in remnant fats from the Wicken Bonhunt assemblage: (a) WKB2; (b) WKB3; (c) WKB7; (d) WKB8; (e) WKB12; (f) WKB15; (g) WKB16, and (h) WKB10.

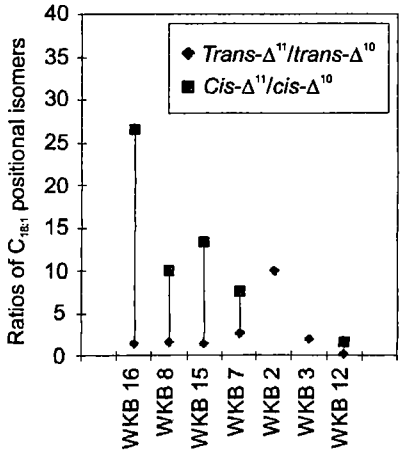


Figure 4.27 Relative abundances of the $C_{18:1}$ Δ^{10} and Δ^{11} *cis*- and *trans*-configured isomers in remnant fats from the Wicken Bonhunt assemblage.

4.3.2.2 Botai, Kazakhstan (early Neolithic)

Extracts from the Botai assemblage, including both absorbed and carbonised surface residues, are all characterised by a higher abundance of the $C_{16:0}$ fatty acid than the $C_{18:0}$ (Fig. 4.28), with a relatively high abundance of the $C_{14:0}$ fatty acid (3 to 7% of total FFA; Table 21, Appendix 3, p. 368). The branched-chain $C_{17:0}$ fatty acid is only present in trace amounts in these samples, whereas the aliphatic $C_{17:0}$ component is present in relatively high abundance (3 to 7% of total FFA). This distribution compares well with the distributions typical of the reference horse fats. With the exception of 'ipot', the Botai extracts all yielded $C_{14:0}/C_{17:0}$ ratios greater than 1.0 (Table 24, Appendix 3, pp. 371-373). The $C_{18:1}$ components in the remnant fats were absent or only present in very minor abundance.

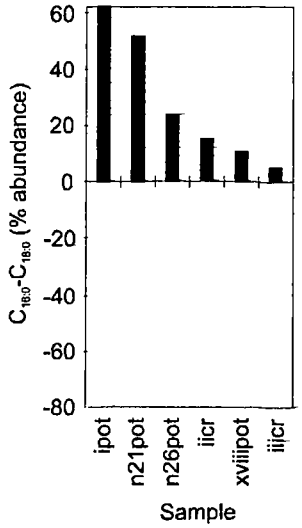


Figure 4.28 Relative abundances of $C_{16:0}$ and $C_{18:0}$ fatty acids in remnant fats from the Botai assemblage. Descriptions of the samples are given in Table 5, Appendix 1 (p. 343).

4.3.3 Prehistoric archaeological sites

4.3.3.1 Yarnton Cresswell field (early-middle Iron Age)

The data from Yarnton Cresswell field mirror that from the flood plain site with a similar distribution of $C_{16:0}/C_{18:0}$ fatty acid ratios (Fig. 4.29; Table 15, Appendix 3, p. 365). Ten out of 25 extracts have a relatively high abundance of the $C_{14:0}$ component. The characteristics of the distributions from the Yarnton flood plain and Cresswell field vessels are distinctively different from the other assemblages studied.

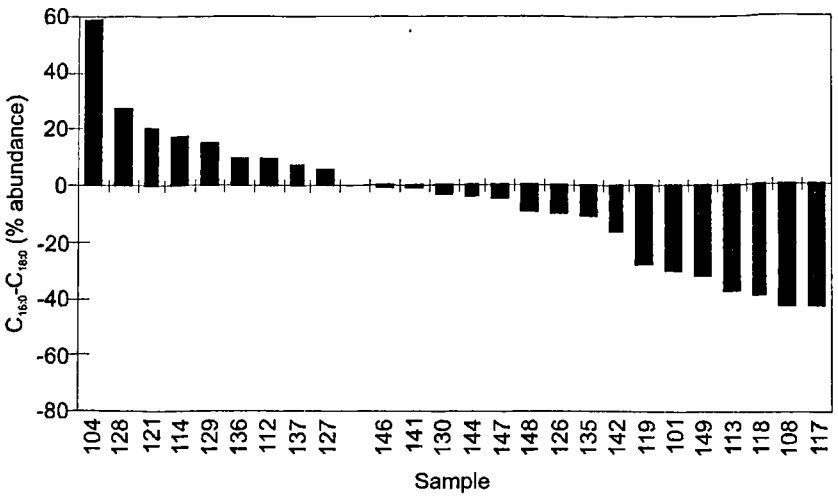


Figure 4.29 Relative abundances of $C_{16:0}$ and $C_{18:0}$ fatty acids in remnant fats from the Yarnton Cresswell field assemblage. Descriptions of the samples are given in Table 6, Appendix 1 (pp. 344-345).

4.3.3.2 Yarnton flood plain (Neolithic-Bronze Age)

Approximately half of the residues from Yarnton flood plain showed a greater abundance of the $C_{16:0}$ fatty acid than the $C_{18:0}$ (Fig. 4.30; Table 16, Appendix 3, p. 366), a much higher proportion of fats with this ratio than seen in any of the other archaeological assemblages studied. Furthermore, the $C_{14:0}/C_{17:0}$ ratio is relatively high (> 2) in 5 out of 14 of the lipid extracts. These distributions of fatty acids are characteristic of horse and cow's milk fats and also pig adipose fats, however, these ratios are not so reliable in the identification of pig fat because of the relative ease with which the composition of pig fats are influenced by their diet. Several remnant fats yielded a relatively low $C_{16:0}/C_{18:0}$ ratio and a high $C_{14:0}/C_{17:0}$ ratio, characteristic of the reference sheep milk fats. The distributions of $C_{18:1}$ isomers have not yet been determined for the Yarnton sites, although many of the extracts contained abundant unsaturated fatty acids.

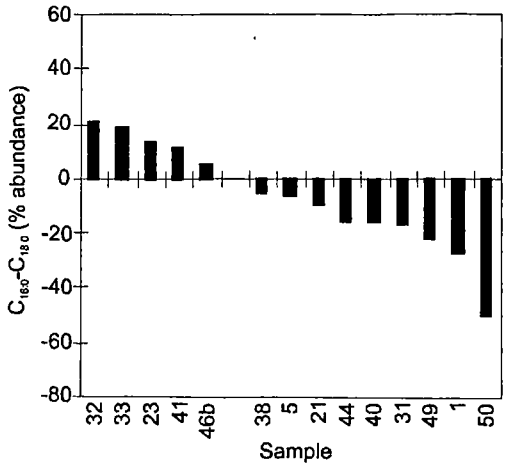


Figure 4.30 Relative abundances of $C_{16:0}$ and $C_{18:0}$ fatty acids in remnant fats from the Yarnton flood plain assemblage. Descriptions of the samples are given in Table 7, Appendix 1 (pp. 346-347).

4.3.3.3 Eton Lake End Road (late Neolithic-Early Bronze Age)

The Eton Lake End Road fatty acid data are surprisingly similar to the Eton Rowing Lake data, with a high number of remnant fats exhibiting greater abundances of the $C_{18:0}$ fatty acid (Fig. 4.31; Table 17, Appendix 3, p. 366). These extracts are dominated by the unusually high abundance of the $C_{18:0}$ component, particularly samples 772, 2041-8 and 1135. The $C_{14:0}/C_{17:0}$ ratios are generally <1, as seen in the West Cotton and Stanwick extracts, except for samples 2163, 865 and 2041-12 (Table 24, Appendix 3, pp. 371-373).

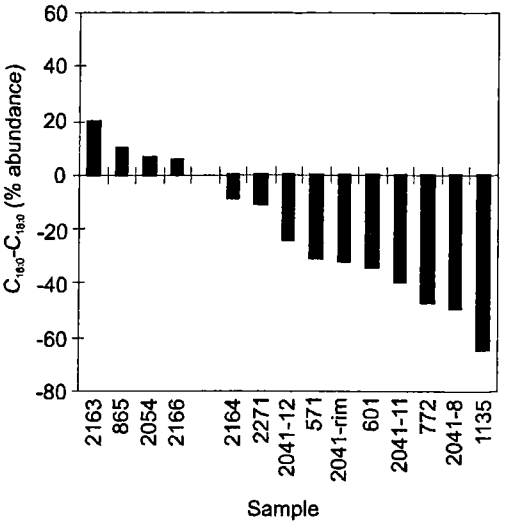


Figure 4.31 Relative abundances of $C_{16:0}$ and $C_{18:0}$ fatty acids in remnant fats from the Eton Lake End Road assemblage. Descriptions of the samples are given in Table 8, Appendix 1 (p. 348).

4.3.3.4 Eton Rowing Lake (early Neolithic)

The fatty acid data from Eton (DBC) mirror that seen for West Cotton and Stanwick with the majority of extracts comprising a greater abundance of the $C_{18:0}$ fatty acid than the $C_{16:0}$ (Fig. 4.32; Table 18, Appendix 3, p. 367). Four of the extracts, DBC7, 8, 13B and 12 have a significantly higher abundance of the $C_{18:0}$ component. The $C_{14:0}/C_{17:0}$ ratios are very low (generally <1) and comparable with the West Cotton and Stanwick data but not the Yarnton data (Table 24, Appendix 3, pp. 371-373). Only samples DBC28, 22, 21, 20 and 3 have $C_{14:0}/C_{17:0}$ ratios >1. Sample 13B contains no $C_{17:0}$, although the $C_{14:0}$ fatty acid is present in abundance (5.5%). The distributions of $C_{18:1}$ positional isomers have not yet been determined for the Eton sites, although many of the extracts contained abundant unsaturated fatty acids.

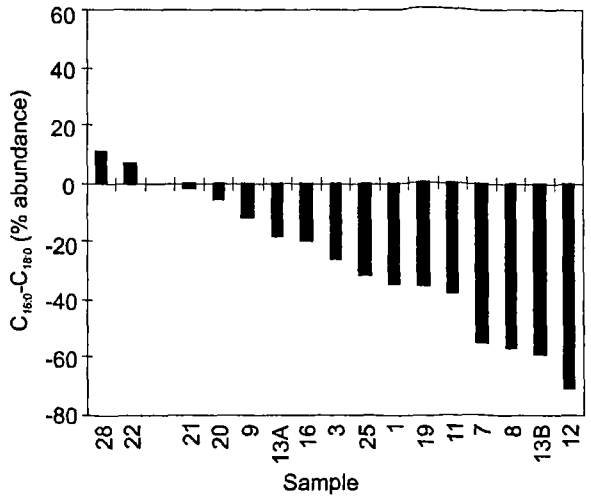


Figure 4.32 Relative abundances of $C_{16:0}$ and $C_{18:0}$ fatty acids in remnant fats from the Eton Rowing Lake assemblage. Descriptions of the samples are given in Table 9, Appendix 1 (p. 349).

4.3.3.5 Upper Ninepence (early-late Neolithic)

All of the extracts from the Upper Ninepence assemblage comprise a greater abundance of the $C_{18:0}$ fatty acid than the $C_{16:0}$ (Fig. 4.33), and this pattern is most marked in sample P5 in which the majority of the saturated fatty acid is $C_{18:0}$ (84%) while $C_{16:0}$ comprises only 9% (Table 19, Appendix 3, p. 367).

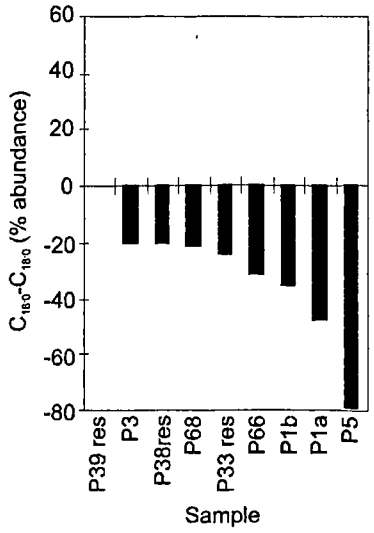


Figure 4.33 Relative abundances of $C_{16:0}$ and $C_{18:0}$ fatty acids in remnant fats from the Walton assemblage. Descriptions of the samples are given in Table 10, Appendix 1 (pp. 350-351).

The proportion of $C_{14:0}$ fatty acid is greatest in P38 and P39 (both carbonised residues), comprising 4.7% and 6.7%, respectively, and less than 1% in samples P5, P1a, P66 and P68 (Table 24, Appendix 3, pp. 371-373). Although the abundance of the straight-chain $C_{17:0}$ component is above 2% in all the extracts, the abundance of the corresponding branched-chain moiety is much less abundant and less than 1% is present in samples P1b, P66 and P68. The differences in the $C_{17:0}$ br/$C_{18:0}$ fatty acid ratios between the remnant fats in P1, P3 and P5 from the Peterborough ware and P33, P39 and P38 from the Grooved ware is shown in Figure 4.34. Clearly, the remnant fats from the different assemblages appear to correlate, with the higher ratio in the Grooved ware samples, predominantly due to the higher abundance of the $C_{17:0}$ br component in these extracts. Due to the lack of mono-unsaturated components in the Walton samples the $C_{18:1}$ positional isomers could not be determined.

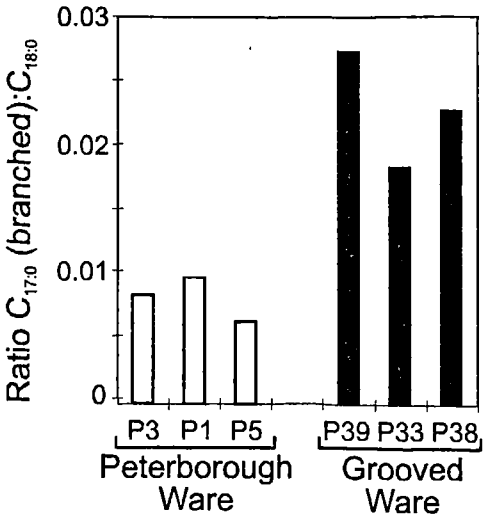


Figure 4.34 Histograms illustrating differences in the ratios of abundance of branched-chain $C_{17:0}$ and straight-chain $C_{18:0}$ fatty acids in Peterborough and Grooved ware extracts. Descriptions of the samples are given in Table 10, Appendix 1 (pp. 350-351).

4.3.4 Ethnographic vessels

4.3.4.1 Vessel A: Dish called *yuvetsi*, containing pork meat, tomato sauce, oregano, salt and pepper.

The predominant fatty acids in this extract are the $C_{14:0}$, $C_{16:0}$, $C_{18:0}$ and $C_{18:1}$, with the $C_{16:0}$ component more abundant than the $C_{18:0}$ in a ratio of 1.8:1 (Fig. 4.35). Minor amounts of straight-chain $C_{15:0}$, $C_{16:1}$ and $C_{17:0}$ and branched-chain $C_{17:0}$ are present, with the branched-chain $C_{17:0}$ fatty acid comprising only 0.4% of the total. There is a greater abundance of $C_{18:1}$ than $C_{16:0}$ in vessel A (Table 20, Appendix 3, p. 368). The $C_{14:0}/C_{17:0}$ ratio (Table 24, Appendix 3, pp. 371-373) shows the high abundance of the $C_{14:0}$ fatty acid (between 3.9 and 17.0%) in all the extracts from the ethnographic vessels, with the exception of vessel C.

The distribution of isomers of the $C_{18:1}$ fatty acid in vessel A are shown in Figure 4.36 (a; Table 4, Appendix 4, p. 375). All the positional and geometric isomers between Δ^9 and Δ^{15} are present with the major isomer being the *cis*-Δ^9, as in all the reference fats studied, and the second major component the *cis*-Δ^{11}. The distributions of the $C_{16:0}$ and $C_{18:0}$ fatty acids (Fig. 4.35) and the abundant *cis*-Δ^{11} (Fig. 4.37) are consistent with that seen in the reference porcine fats (Figs. 4.10 and 4.12), however this ethnographic extract also

contains *cis*- and *trans*-isomers in positions Δ^{10}, Δ^{12}, Δ^{13}, Δ^{14} and Δ^{15} which are absent or present in very low abundance in the reference porcine fats.

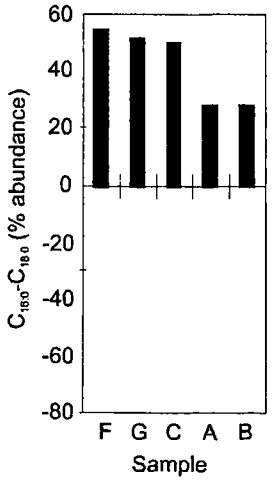


Figure 4.35 Relative abundances of $C_{16:0}$ and $C_{18:0}$ fatty acids in remnant fats from the ethnographic vessels. Descriptions of the samples are given in Table 11, Appendix 1 (p. 352).

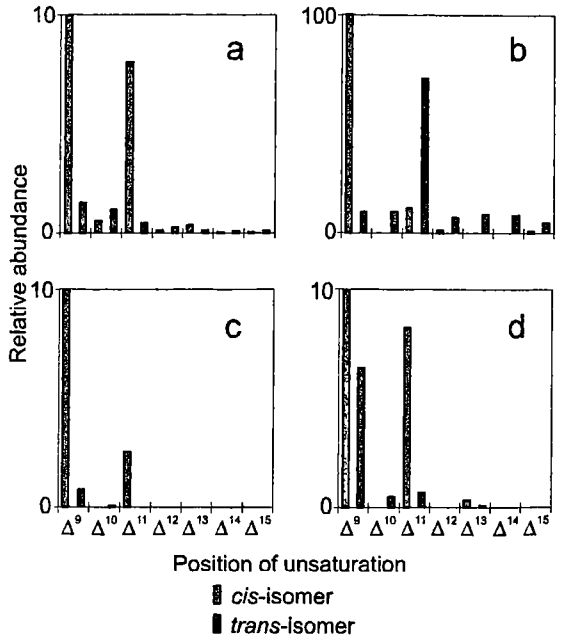


Figure 4.36 Distributions of positional and geometric isomers of $C_{18:1}$ in remnant fats from the ethnographic vessels: (a) vessel A; (b) vessel B; (c) vessel C, and (d) vessel F.

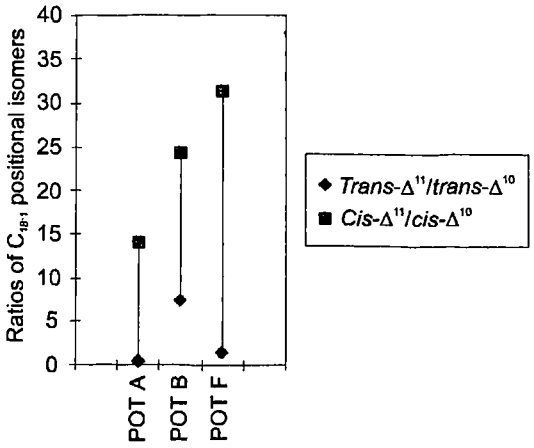


Figure 4.37 Relative abundances of the $C_{18:1}$ Δ^{10} and Δ^{11} *cis*- and *trans*-configured isomers in remnant fats from ethnographic vessels A, B and F.

4.3.4.2 Vessel B: Storage of cheese, milk (with yeast and salt) and used to preserve grapes with hardaki (mustard seed).

The free fatty acid components in vessel B comprise mainly the $C_{14:0}$, $C_{16:0}$ and $C_{18:0}$ components, with minor $C_{12:0}$, $C_{15:0}$, $C_{16:1}$, $C_{17:0}$, $C_{18:1}$, $C_{20:0}$ and branched-chain $C_{15:0}$ and $C_{17:0}$ (Table 20, Appendix 3, p. 368). The ratio of $C_{16:0}$ to $C_{18:0}$ is 1.8:1 (Fig. 4.35). Although the ratio of the major saturated fatty acids is similar in extracts from vessels A and B, there is significantly less $C_{18:1}$ in vessel B although the shorter-chain $C_{12:0}$ fatty acid is present in the latter vessel. There is a greater abundance of the odd-carbon number and branched-chain $C_{15:0}$ and $C_{17:0}$ components in vessel B, comprising a total of 6.3%.

The $C_{18:1}$ positional isomers in vessel B include the *cis*-Δ^9 as the major isomer, followed by the *trans*-Δ^{11} [Fig 4.36 (b)]. The effect of decay is evident since the proportion of the *cis*-Δ^9 isomer comprises only 43% of the total $C_{18:1}$, whereas in the fresh reference dairy fats analysed the *cis*-Δ^9 comprised 89% of the total. With the exception of *cis*-Δ^{11}, the $C_{18:1}$ isomers are present only in the *trans*-form. The proportion of *cis*-Δ^{11} is likely to have been significantly greater in the fresh fat. The ratios of *cis*- and *trans*- Δ^{10} and Δ^{11} isomers plotted in Figure 4.37 are comparable with those in the reference cow's milk fat.

4.3.4.3 Vessel C: Storage of olive oil.

The fatty acid distribution is very similar to that seen in the reference olive oil, comprising largely of $C_{16:0}$, $C_{18:0}$ and $C_{18:1}$, with the latter component comprising 68% of the total

(Table 20, Appendix 3, p. 368). No $C_{14:0}$ or shorter-chain fatty acids were detectable, as was the case in the reference olive oil. The aliphatic $C_{17:0}$ comprised less than 1% and the branched-chain $C_{17:0}$ was only present in trace amounts (Table 24, Appendix 3, pp. 371-373). The $C_{16:0}/C_{18:0}$ ratio is 3:1. There are few positional isomers of the $C_{18:1}$ present, with the *cis*-Δ^9 comprising 97% of the total fatty acid and the other isomers including *trans*-$\Delta^{9,10}$ and *cis*-Δ^{11} [Fig. 4.36 (c)]. There are two *trans*-configured fatty acids present which were not detected in the reference olive oil.

4.3.4.4 Vessel F: Storage of a mixture of pork fat, pork meat, salt, paprika, black pepper, oregano, onions and bahari (spice).

This extract exhibits a range of fatty acids, including abundant $C_{14:0}$, $C_{16:0}$ and $C_{18:0}$ and $C_{18:1}$, with trace amounts of the branched-chain $C_{17:0}$ and less than 1% of the straight-chain $C_{17:0}$ component. The $C_{16:0}$ is significantly more abundant than the $C_{18:0}$, in the ratio of 3.3:1 (Table 20, Appendix 3, p. 368). The $C_{18:1}$ isomers include abundant *cis*-Δ^9 (86%) and *trans*-$\Delta^{10,11,13}$ and *cis*-$\Delta^{11,13}$, with the *cis*-Δ^{11} dominating the minor components [Fig. 4.36 (d)]. The ratios of the isomers (plotted in Fig. 4.37), the abundance of the *cis*-Δ^{11} isomer, the generally small range of positional and geometric isomers and the low abundance of the $C_{17:0}$ fatty acids is consistent with that seen in reference porcine fats.

4.3.4.5 Vessel G: Milk and cheese storage.

The fatty acids in the extract from vessel G range from $C_{8:0}$ through to $C_{18:0}$, with $C_{16:0}$ the major component (Table 20, Appendix 3, p. 368). The ratio of $C_{16:0}$ to $C_{18:0}$ is 3:1 and the $C_{14:0}$ component is highly abundant, comprising 14.6% of the free fatty acids, compared to 3% in vessel A, 7% in vessel B and 5% in vessel F. The $C_{15:0}$ and $C_{17:0}$ straight- and branched-chain components are also abundant, comprising a total of 6.3%. This compares well with the residue from vessel B, also reported to have been used for storing dairy products, in which these components also comprise 6.3% of the total free fatty acids. The $C_{18:1}$ positional isomers in vessel G were not characterised.

4.3.5 Siberian horse tissues

The fatty acids comprised $C_{14:0}$, $C_{16:0}$, straight- and branched-chain $C_{17:0}$, $C_{18:0}$ and mono- and dienoic C_{18} (Table 22, Appendix 3, p. 368). The preservation of these horses was such

that the unsaturated C_{18} components have been preserved to a large extent and still comprise between 8% and 20% of the fatty acid. It is very unusual to see such abundances of unsaturated moieties in archaeological samples. In this case, the permafrost burial appears to have minimised the rate of microbial and enzymatic decay, however, the presence of the hydroxy acid shows that some diagenetic alteration had taken place. The relative abundances of fatty acids present are given in Table 22, Appendix 3 (p. 368). The higher abundance of the $C_{16:0}$ than the $C_{18:0}$ fatty acid is very characteristic of horse fat, with skin, sacrum and chest samples comprising a ratio of 3.4:1, 3.1:1 and 1.4:1, respectively. The abundance of the $C_{14:0}$ fatty acid is particularly high in the skin and sacrum samples, comprising 7.5 and 11.1% of the total, respectively. Positional and geometric isomers of the $C_{18:1}$ component were quantified for skin and sacrum samples and the data are shown alongside the reference horse fats in Figure 4.11 for comparison. The distribution of isomers in the Siberian horse fats is consistent between samples, but differs significantly from the distribution in the modern reference horse fats (Table 3, Appendix 4, p. 375). There are a greater range of isomers in the Siberian fats, from Δ^9 to Δ^{15}, and with the *cis*- and *trans*-Δ^9, ^{10} and Δ^{11} isomers in relatively high abundance. However, in the reference horse fats only the *cis*-Δ^9 and *cis*-Δ^{11} are present in abundance. Furthermore, whereas the Δ^{11} isomer is predominantly in the *cis*-form in the reference horse fats, it is present mainly in the *trans*-form in the Siberian horse fats. Figure 4.38 shows the ratios of the *cis*-Δ^{11}:Δ^{10} and *trans*-Δ^{11}:Δ^{10} positional isomers are approximately equal in the Siberian horse fats. The distribution of the *cis*- and *trans*-Δ^{10} and Δ^{11} isomers in the Siberian horse fats bear a remarkable similarity to those in the reference deer fats from animals raised on natural pasture, rich in herbs. The deer are known to be selective grazers, particularly partial to herbs and it is known that herbs would also have formed part of the diet of the Siberian horses.

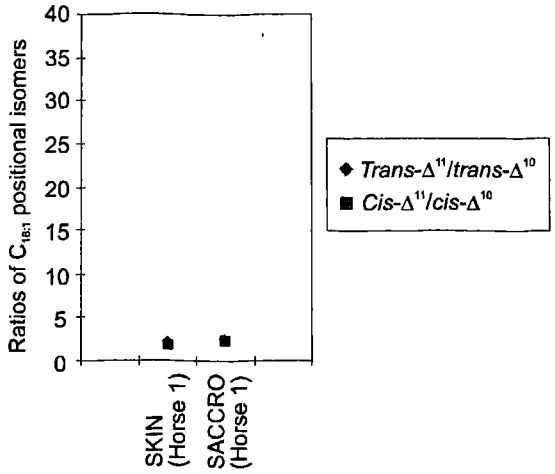


Figure 4.38 Relative abundances of the $C_{18:1}$ Δ^{10} and Δ^{11} *cis*- and *trans*-configured isomers in the Siberian horse fats.

4.4 Discussion

Detailed analysis of the animal reference fats has enabled a large set of fatty acid compositional data to be obtained on animals raised on known C_3 diets. The only detailed information on the distributions of $C_{18:1}$ positional and geometric isomers found in the literature related to cow adipose and butter fat, which has been discussed, however the diets of the animals concerned were not stated. Discussed here are the principal trends which have emerged from the studies of modern reference and archaeological fats.

Trends observed in the overall fatty acid distributions in modern reference fats

The ratios of abundance (%) of $C_{16:0}$ to $C_{18:0}$ fatty acids in the reference fats has clearly shown the predominance of the $C_{16:0}$ in the cow adipose and milk fats and in all of the non-ruminant and pseudo-ruminant fats. The cows' milk generally contained a greater proportion of $C_{16:0}$ to $C_{18:0}$ than cow adipose fat (with one anomaly). Lamb fats and some of the deer fats showed a greater abundance of the $C_{18:0}$ fatty acid. The published data are inconsistent in this respect, with examples of internal and subcutaneous fats varying considerably in the distribution of the major saturated fatty acids, possibly reflecting the contribution of cereals to the diet. The sheep milk fat contained significantly less $C_{16:0}$ compared to $C_{18:0}$ than in cow's milk fat which may be diagnostic, although to date only one sample of sheep milk has been quantified. The shorter-chain fatty acids ($<C_{14}$) predominated in the milk fats. The deer fats are inconsistent in composition, although the

renal (kidney) fats both comprise less $C_{16:0}$ than $C_{18:0}$ as does the cow renal fat. The fatty acid composition of rumen bacteria, reported by Ifkovits and Ragheb (1968) indicate that the rumen micro-organisms are the source of odd-carbon number and branched-chain fatty acids found in the milk and body fat of ruminant animals. The straight- and branched-chain $C_{17:0}$ components were present in approximately equal abundance in ruminant fats, while in horse fats 75% of the C_{17} fatty acids were solely straight-chain. There was a higher proportion of saturated fatty acids in ruminant fats, which comprised mainly $C_{14:0}$, $C_{16:0}$ and $C_{18:0}$, whilst non-ruminant fats comprise mainly $C_{16:0}$ and C_{18} mono- and polyunsaturated acids. The proportion of $C_{14:0}$ fatty acid appeared to be greater in ruminant milk fats than in ruminant adipose fats, for example comprising a mean of 10.8% in cow's milk and only 3.8% in cow adipose. It is well known that the composition of the depot fats of ruminant species is largely unaffected by dietary changes or the feeding of large amounts of unsaturated fats or oils (Church, 1988, and references therein) and this factor is significant in the study of fats from ancient animals of which the diet is unknown. The ratio of $C_{16:0}$ to $C_{18:0}$ fatty acids was quite consistent between non-ruminant animals, with the $C_{16:0}$ fatty acid predominating in fish oil, horse, chicken and goose fats. The absence of branched-chain and odd-carbon number fatty acids clearly distinguishes the non-ruminant from the ruminant modern reference fats, however $C_{17:0}$ fatty acids are present in the horse fats possibly due to their extended stomach which houses some fermentation.

Trends observed in the distributions of $C_{18:1}$ positional and geometric isomers in modern reference fats

The results of $C_{18:1}$ positional isomer analysis has shown tentative distinctions can be drawn between modern reference sheep and cow's milk on the basis of the ratio of the *cis*- and *trans*-Δ^{11} isomers. Sheep and cow adipose are also distinguished by a greater proportion of *trans*-Δ^{11} than *cis*-Δ^{11}. Cow's milk and cow adipose fats can be distinguished by comparing the differences between the *trans*-Δ^{11}:Δ^{10} and *cis*-Δ^{11}:Δ^{10} ratios. Numerous positional and *cis*- and *trans*-configured isomers of the unsaturated C_{18} acid have been found in ruminant fats, whereas in omnivore fats the range is more restricted, thus ruminant and non-ruminant fats from animals fed pure C_3 diets are distinguishable on the basis of the abundance of *cis*- and *trans*-positional isomers ranging from Δ^{10} to Δ^{15} in ruminant fats. In all ruminant fats, with the exception of the Δ^9 isomers, where the *cis*- always dominates,

the components with the *trans*-configuration are more abundant than their *cis*-counterparts. In all non-ruminant fats the *cis*-Δ^{11} is the most abundant after the *cis*-Δ^{9} component. Mixtures of ruminant and non-ruminant fats may be identifiable according to the proportion of different positional isomers present. Pig, chicken and goose fats are indistinguishable on the basis of their positional isomer distributions.

There is a distinctive pattern in the distribution of positional and geometric isomers, which is likely to result from the desaturase activity of microbes rather than diet in ruminant animals. In studies of the actions of intestinal microflora (*Eubacterium*), Eyssen and Parmentier (1974) recognised that the formation of a conjugated *trans*-$\Delta^{9,11}$ or *trans*-$\Delta^{10,12}$-isomer in the biohydrogenation of $C_{18:2}$ due a shift of one of the double bonds in the *cis* fatty acid, resulted in the formation of $C_{18:1}$ identified (albeit tentatively) as *trans*-Δ^{11}, or *trans*-vaccenic acid. The biohydrogenation of $C_{18:2}$ in sheep rumen by *Butyrivibrio fibrisolvens* has been reported to proceed following the same pathway (Kepler *et al.*, 1966), and this is likely to be the reason why the *trans*-vaccenic acid is relatively abundant in the ruminant reference fats. The *cis*-Δ^{11} component is more abundant in ruminant milk fats, possibly due to the fact that a proportion of the C_{18} components from the diet routed directly to milk production are only partially hydrogenated in the rumen. Duncan and Garton (1967) report that since monogastric animals, e.g. pigs, cannot modify unsaturated fatty acids in the same way as ruminant animals, *trans* acids tend to be lower in their depot fats, the only possible source of these acids being the diet of the animal. The concern is that the $C_{18:1}$ of fats of non-ruminant animals given supplements (e.g. scraps/waste) to their diet in antiquity, may reflect the range of $C_{18:1}$ isomers in the diet, therefore complicating archaeological interpretations.

Trends in the archaeological data

The majority of samples (76%) from West Cotton appear from the ratio of $C_{16:0}$ and $C_{18:0}$ fatty acids to derive from ovine fats, which correlates well with the high proportion of sheep bone excavated from the site. Only three of the samples appear to represent non-ruminant fats, due to their lack of $C_{17:0}$ branched-chain components. The abundance of the *trans*-Δ^{11} component indicates that the majority of the remnant fats derive from ruminant

fats. Furthermore, a number of samples comprised relatively high abundances of the $C_{14:0}$ fatty acid indicative of dairy fats.

The remnant fats from Stanwick all appear to derive from ruminant animals. The $C_{16:0}/C_{18:0}$ ratio indicates that the majority of the residues are derived from ovine fats, whilst three residues may be derived from bovine dairy fats since the $C_{16:0}/C_{18:0}$ ratio is comparable to that of the reference bovine fats and the high % of $C_{14:0}$ fatty acid indicates dairy fats. In addition, the $C_{18:1}$ *trans*-configured isomers are dominated by the Δ^{11} component in the majority of samples. It is reassuring that analyses of different sherds believed to be part of the same vessel (ST208) give comparable data, indicating that the chemical characteristics of the remnant fats are representative of the original lipid profile.

Wicken Bonhunt is believed to have been a centre for the breeding of pigs and distribution of their meat/fat to outlying hinterlands in the Middle Saxon period and, indeed, some of the characteristics of the fatty acid distributions do reflect the use of these vessels in the processing of porcine fats. The low abundance of the branched- and straight-chain $C_{17:0}$ components in samples WKB3, 8 and 16 is consistent with the reference non-ruminant fats as is the relatively low abundance of the $C_{14:0}$ fatty acid, indicated by the $C_{14:0}/C_{17:0}$ ratio. The similarity is particularly noticeable in the distributions of $C_{18:1}$ fatty acid isomers, since all the samples, with the exception of WKB12 exhibit the distinctive signal seen for the reference non-ruminant fats. The ratio of $C_{16:0}$ and $C_{18:0}$ fatty acids does not support the assignment as porcine fats, since the $C_{18:0}$ is generally higher than the $C_{16:0}$ fatty acid in the archaeological extracts, whereas in the reference pig fats the $C_{16:0}$ is distinctly more abundant than the $C_{18:0}$. However, the $C_{16:0}/C_{18:0}$ ratio may have been altered during decay.

It was originally anticipated that the residues from Botai sherds would reflect characteristics of the reference horse fats, and indeed, the high proportion of $C_{16:0}$ compared to the $C_{18:0}$ fatty acid in the Botai extracts is very similar to the reference horse fats. In addition, the residues comprise relatively high proportions of the straight-chain $C_{14:0}$ and $C_{17:0}$ fatty acids and minor amounts of the branched-chain $C_{17:0}$ also characteristic of the reference horse fats. The Siberian horse fats are also comparable in all respects, except in the distribution of $C_{18:1}$ positional isomers. It is possible that the additional $C_{18:1}$

components may have arisen through microbial desaturase activity during decay, although the preservation of the highly abundant unsaturated components would suggest that microbial activity was low.

The Walton residues appear to derive from ovine fats due to the ratio of $C_{16:0}/C_{18:0}$ fatty acids, and the high proportion of $C_{14:0}$ in P38 and P39 is indicative of dairy fats. However, the low abundance of branched-chain $C_{17:0}$ in P1b, P66 and P68 is indicative of non-ruminant fats. These data are inconclusive, but the fatty acid distributions may have been affected by decay since only free fatty acids were detected in samples P1a or P5 with no intact acyl moieties remaining. These samples comprised the lowest $C_{16:0}/C_{18:0}$ ratio, and less than 1% $C_{14:0}$ fatty acid, possibly due to the preferential loss of the shorter-chain fatty acids through dissolution.

It appears that patterns have begun to emerge in the data from Yarnton and Eton: Yarnton flood plain and Cresswell field vessels are characterised by a greater number of extracts with a high abundance of the $C_{16:0}$ compared to the $C_{18:0}$ fatty acid and also a number of extracts with high $C_{14:0}/C_{17:0}$ ratios; conversely, the pottery extracts from the two Eton sites are characterised by extracts containing a relatively high abundance of the $C_{18:0}$ fatty acid and low $C_{14:0}/C_{17:0}$ ratios.

Observations from the ethnographic data

The residues from ethnographic vessels A and F were found to be very similar to the reference porcine fats, with high $C_{16:0}/C_{18:0}$ ratios and abundant $C_{18:1}$ reflecting the high unsaturation of pork fat. The $C_{14:0}/C_{17:0}$ fatty acid ratio for the ethnographic pork residue in vessel A was 3.3, comparing well with the ratio in the reference fat of 3.97. The same ratio in vessel F was 7.37, reflecting the very low abundance of $C_{17:0}$ in the pork fat. Furthermore, the *cis*-Δ^{11} was present in high abundance, characteristic of non-ruminant fats. The only anomaly between the ethnographic and reference pork fat was the abundance of the isomers in positions Δ^{10}, Δ^{12}, Δ^{13}, Δ^{14} and Δ^{15} which were absent or present in very low abundance in the reference porcine fat. The accumulation of dietary fatty acids directly into adipose tissue of omnivores explains the appearance of branched-chain components in the archaeological pig fats. They are most likely of exogenous origin, directly related to the

quantity of dairy products or ruminant meat scraps consumed, as shown in studies of human depot fat (Shorland *et al.*, 1969; Jacob and Grimmer, 1967). The other ingredients used in the preparation of the pork dishes in the ethnographic vessels, i.e. tomato sauce, paprika, oregano, onions, bahari (spice), salt and pepper, left no detectable lipid residue.

The presence of the short-chain $C_{12:0}$ component, the abundance of odd-carbon number and branched-chain fatty acids and the distribution of positional isomers is consistent with the storage or processing of milk or cheese in ethnographic vessels B and G. The reference dairy fats are characterised by a greater abundance of the *cis*-Δ^{11} than that seen in the extract from vessel B, which is probably due to the greater susceptibility of the *cis*-configured isomers to decay. The ratio of $C_{16:0}$ and $C_{18:0}$ fatty acids is consistent with that seen in the reference cow's milk. This is strongly supported by the ratios of $C_{18:1}$ isomers in the Δ^{10} and Δ^{11} positions. The effect of decay is seen in the overall low abundance of the monounsaturated C_{18} components and the preferential loss of the *cis*-configured $C_{18:1}$ isomers. Unsaturated moieties are likely to be involved in reactions such as reduction and hydration; hydration may result in the formation of e.g. 10-hydroxyoctadecanoic acid, whereas reduction could result in an increase in the proportion of the $C_{18:0}$ fatty acid present. The latter has serious implications for relying on the use of fatty acid ratios in distinguishing between remnant animal fats.

The identification of milk fats has, in the past, relied on detecting the presence of short-chain, saturated fatty acids containing less than twelve carbon atoms ($<C_{12}$). In fresh milk the shorter-chain components ($C_{4:0}$-$C_{12:0}$) typically account for up to 20% of the total fatty acid content of milk fat (McDonald *et al.*, 1988), however, no fatty acids lower than $C_{12:0}$ have been detected in the ethnographic milk fat residues. The lower abundance of the diagnostic short-chain fatty acyl moieties in the ethnographic vessels can be attributed to their preferential loss from sherds compared to their long-chain counterparts during burial on two accounts. Firstly, it is well established that the short-chain fatty acid moieties are located primarily at the *sn*-3 position in the triacylglycerols (Brockerhoff *et al.*, 1966; Parodi, 1979, 1982; Section 1.6.7) and will be more susceptible to enzymatic and chemical hydrolysis due to reduced steric hindrance effects. Even as early as 1937 Balls and co-workers noted that "the ease at which lower fats are hydrolysed at low temperatures may

account for difficulties in preserving fats such as butter". Secondly, once released, the short-chain fatty acids are significantly more water-soluble than their long-chain counterparts. Figure 4.39 is a plot of the relationship between the solubility (in water at neutral pH) and the carbon chain-length of the major saturated fatty acids of milk; clearly, the solubility decreases markedly with increasing chain-length. These two factors alone are sufficient to explain the differences seen between the fatty acid distributions in the fresh milk fat and lipid extracts of archaeological vessels. Many other factors, e.g. degree of waterlogging, pH, temperature, soil type, nature of ceramic fabric, etc., will further influence the rate and extent of fat degradation. However, the inherent differences in the chemical properties of dairy and adipose fats, i.e. the behaviour of shorter-chain length fatty acyl moieties in the former, will be an overriding factor resulting in comparable free fatty acid distributions in diagenetically altered dairy and adipose fats (discussed further in Chapter 7).

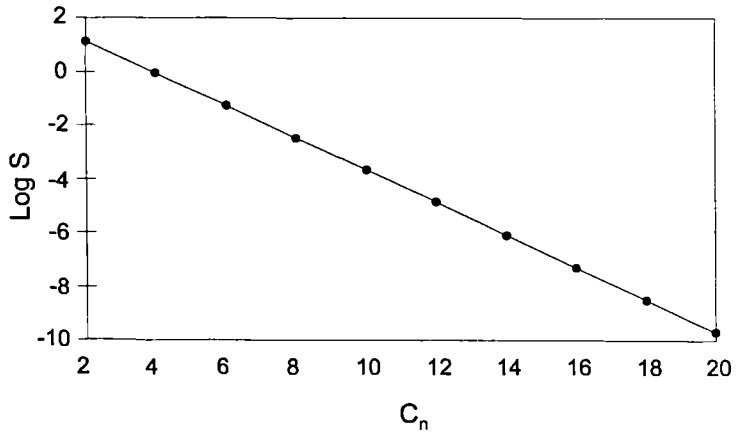


Figure 4.39 Plot of the relationship between the solubility (in water at neutral pH) and the carbon chain length (C_n) of saturated fatty acids, according to the expression: log S=c + *k*n, where c=2.32 and *k*=-0.60 (Bell, 1973).

The residue in ethnographic vessel C is characteristic of degraded olive oil, with a distribution of both saturated and mono-unsaturated components comparable to the reference olive oil including, notably, the lack of the $C_{14:0}$ fatty acid. The proportion of the $C_{18:1}$ *cis*-Δ^9 component was lower than in fresh olive oil due to the relatively high susceptibility of the *cis*-configured components to decay.

On a more general note, the effect of decay in both the ethnographic and archaeological residues is reflected in the low abundance of monounsaturated components and complete lack of polyunsaturated components in the solvent extracts. The fatty acid compositions of the less well preserved residues from Walton and the decay of the shorter-chain fatty acids in the ethnographic milk fats suggest that the preferential decay of shorter-chain components needs to be considered when using fatty acid ratios in assigning the origins of animal fats. The *cis*-configured isomers have clearly suffered from decay to a much greater extent than the *trans*-isomers, with only the *cis*-Δ^9, the most abundant $C_{18:1}$ component in fresh fats, present in many of the archaeological extracts. For this reason, $C_{18:1}$ *cis/trans* ratios have not been considered in characterising the archaeological fats. Reasons for the greater susceptibility of the *cis*-configured isomers have been discussed in Section 4.1.3.

CHAPTER 5
Triacylglycerol distributions

5.1 Variation in triacylglycerol composition of fats and oils

5.1.1 The structure of triacylglycerols

The molecular structure of a triacylglycerol is given in Section 1.6.7. Triacylglycerols occur with characteristic arrangements of fatty acyl moieties on the glycerol backbone, partly due to an enzyme specificity associated with their biosynthesis which occurs as a result of hydrolysis and re-esterification both at the intestinal level and at the site of utilisation, e.g. liver, muscle, adipose tissue, etc. The stereospecific analysis of fatty acids comprising triacylglycerols has been widely reviewed (e.g. Christie, 1978; Anderson *et. al.*, 1970), and it is now generally accepted that the location of particular fatty acids on the glycerol backbone conform to the rules initially proposed by Brockerhoff (1966).

It has been speculated that in order to minimise changes to the fatty acid composition of body fats, e.g. in response to dietary alteration, compensatory modifications occur in the rates of biosynthesis and oxidation of specific fatty acids (Carroll, 1965). Systemic alterations such as these would suggest that animals are able to maintain a constant unsaturated to saturated fatty acid ratio, thus maintaining homeostasis of the physical properties of their depot fat (Beare-Rogers, 1970). Based upon these factors it is hypothesised that the range of different triacylglycerol distributions which we observe in archaeological fats may not be the result of different degrees of preservation, but may be reflecting characteristics specific to different animal types. Encouragingly, several studies have also reported non-random placement of fatty acids of specific chain-length in milk fat triacylglycerols (Breckenridge and Kuksis, 1968; Robinson and MacGibbon, 1998) thus validating the use of intact triacylglycerol distributions in distinguishing between species.

5.1.2 The occurrence of intact triacylglycerols in archaeological contexts

Fatty materials found in graves and buried in soil have usually been completely transformed from their original constitution, often described as resembling 'adipocere', which has been defined as a mixture of fatty acids, with palmitic acid the principal constituent (Thornton *et al.*, 1970). Investigations of fatty materials from a number of archaeological finds including a Roman phial (Jáky *et al.*, 1964), 'bog butters' dated to *ca* AD 140-346 discovered in peat bogs in Scotland and Ireland (Morgan *et al.*, 1970), 1000-year-old fat samples from a midden in North Western Canada (Morgan *et al.*, 1983) and a

submerged wreck (Morgan *et al.*, 1992) found abundant free fatty acids but no intact acyl lipid components. Similarly, only hydrolysed lipid components were detected in the tissues of seven Dutch bog bodies (Evershed, 1990), or Lindow Man, recovered in a peat near Wilmslow, Cheshire, UK, where hydrolysis may have been accelerated due to prolonged submergence in the acidic burial environment (Evershed and Connolly, 1988). In contrast, great success in retrieving abundant intact triacylglycerols has been had by the solvent extraction of absorbed and carbonised residues associated with archaeological pottery vessels. High temperature gas chromatographic analyses has revealed the preservation of intact acyl lipids derived from animal fats processed in a Late Saxon cooking vessel from West Cotton in Northamptonshire. The confident identification of the residue as a degraded animal fat was based upon comparison of the distribution of partially hydrolysed acyl lipids and free fatty acids with that of a laboratory degraded bovine fat (e.g. Evershed *et al.*, 1992b). Intact triacylglycerols have been identified in the carbonised surface deposits adhering to Neolithic vessels from the Lake dwelling site of Chalain, Jura, France (Regert *et al.*, 1998), where their preservation in such high abundance is attributed to the permanently waterlogged burial environment, as well as the physical protection provided by entrapment in the clay matrix of the potsherds.

5.1.3 Separation and structural elucidation of complex natural mixtures of triacylglycerols and their use in determining origin

In recent years, the development of analytical techniques enabling the separation of complex natural mixtures has significantly advanced the structural elucidation of triacylglycerols. Laakso (1996) has reviewed the use of various techniques, including high-performance liquid chromatography, gas chromatography and supercritical fluid chromatography in the separation of species, and discusses the advantages of mass spectrometry for structural elucidation. Myher *et al.* (1988) have argued that the use of polar capillary GC is a superior technique to reversed-phase HPLC in the analysis of complex mixtures of triacylglycerol components, e.g. those found in milk fats, because of the ease of quantification provided by the flame ionisation detector and the much larger number of theoretical plates for the resolution of closely related molecular species. However, the use of reversed-phase HPLC and GC enabled the identification of 181 molecular species of triacylglycerol, including 79 saturated, 44 monounsaturated and 58

polyunsaturated moieties (Ruiz-Sala *et al.*, 1996). This detailed distributional analysis revealed clear differences between the triacylglycerol composition of ewe, cow and goat milk based upon comparisons of groups of triacylglycerols with the same molecular features, i.e. short, medium and long-chain fatty acids. Ewe milk was found to be much richer in short- and medium-chain triacylglycerols, while cow's milk comprised more abundant long-chain and unsaturated triacylglycerols. Studies have succeeded in drawing distinctions between depot fats of different species by Kagawa *et al.* (1996) and Brockerhoff *et al.* (1968), the effect of diet (Smith *et al.*, 1998) and also tissue location (Christie and Moore, 1971) on the total composition and positional distribution of fatty acids in triacylglycerols.

The use of high temperature gas chromatography (HTGC) using a non-polar methylsilicone type DB1 stationary phase is an efficient, reproducible method of separating mixtures of triacylglycerols, by which resolution is primarily on the basis of the differences in molecular weights (i.e. the combined number of acyl carbon atoms). Triacylglycerols can be clearly resolved into acyl carbon number groups which do not overlap the preceding or following groups and the use of on-column injection avoids problems of discrimination between components of high and low boiling points. This technique has been previously used in the analysis of triacylglycerols in organic residues from archaeological pottery by Evershed *et al.* (1990). This work represented the first application of HTGC analysis to samples of archaeological origin. Detailed compositional information for high molecular weight components and partially hydrolysed acyl lipids was obtained.

The compositional analysis of a large number of archaeological extracts by HTGC has been reported by Charters (1996) in a study of total lipid extracts from late Saxon/early medieval vessels from West Cotton, Northamptonshire. The ranges of triacylglycerol moieties of equal acyl carbon number were considered in making tentative distinctions between remnant animal fats of different origins. The preliminary data obtained stimulated the more in-depth analyses which have been carried out as part of this thesis.

5.1.4 Decay and transformations of triacylglycerols

In general the apolar nature of lipids confers on them hydrophobic properties which make them relatively resistant to dissolution in aqueous media. These properties also contribute to the preservation of organic components absorbed within the pores of ceramic vessels. However, in certain burial conditions protection within the ceramic matrix is not sufficient to prevent the decay of intact acyl lipids, for example by chemical or enzymatic hydrolysis and oxidation (Evershed *et al.*, 1992a; Sections 7.1.2 and 7.1.3).

A study by Ahmad and Bahl (1946) observed that certain fats were more easily hydrolysed than others; pig lard being the easiest to hydrolyse followed by beef, sheep and goat fats. The fermentation of natural fats was investigated by Balls *et al.* (1937) using pancreatic lipase to determine the ease by which different glycerides are hydrolysed. It was discovered that glycerides containing the C_7 to C_{10} acids appear to undergo hydrolysis most readily. The ease at which lower fats are hydrolysed at low temperatures may account for the difficulties in preserving fats such as milk and butter which contain abundant short-chain fatty acids. Hydrolysis of the higher but not the lower, saturated triacylglycerols was dependant on temperature, with the higher components (e.g. trimyristin, tripalmitin and tristearin) regarded as outstanding in their resistance to lipolysis. It was also recognised that the monounsaturated C_{18} components of triacylglycerols abundant in natural fats and oils are as easily hydrolysed as the shorter-chain fatty acids. This is attributed to the C_9=C_9 structure acting like a saturated glyceride of the C_7 to C_{10} range rather than a C_{18} acid. Badings and Neeter (1980) also found that short-chain fatty acids are preferentially cleaved from the glyceride backbone, and is thought to occur due to the higher polarity of their esters. Once one fatty acid has been lost, the complete hydrolysis of acyl lipids occurs more rapidly than for intact triacylglycerols (Davídek *et al.*, 1990).

Furthermore, it is well-established that short-chain fatty acid moieties are located primarily at the *sn*-3 position in the triacylglycerols of natural fats (Brockerhoff *et al.*, 1966; Parodi, 1979, 1982) and due to reduced stearic hindrance effects will be more susceptible to enzymatic and chemical hydrolysis. Positional location and molecular association may also be pertinent to the resistance of long-chain polyunsaturated fatty acids to peroxidation,

although the results of various studies appear contradictory (Myher *et al.*, 1996, and references therein).

Alterations caused to the structures of lipids during the original processing and storage of foodstuffs should not be underestimated. Heat treatments during food processing accelerate hydrolysis in water (Davídek *et al.*, 1990) and the presence of natural lipolytic enzymes, and lipases produced by moulds and airborne bacteria can all contribute to the hydrolytic rancidification of foodstuffs. Oxidation reactions also occur frequently in food materials and are discussed in Section 7.1.3 and reviewed in Davídek *et al.* (1990). The oxidation of polyunsaturated fatty acids or esters containing polyunsaturated acyl moieties occurs readily in aqueous solution and is catalysed by the presence of oxygen, producing water soluble products which have been widely studied due to their effect on the metabolism, structure and growth of cells in biological systems (Schauenstein, 1967). The conversion of triacylglycerols through allylic radical intermediates to the cross-linked product is well documented classical organic chemistry and has been extensively studied, e.g. in the formulation of drying oils and paints. Polyunsaturated vegetable oils, e.g. linseed, composed of C_{16} to C_{26} polyunsaturated fatty acids undergo free radical oxidative crosslinking in air, especially in sunlight and in the presence of transition metals (Kirschenbauer, 1960).

5.1.5 The characterisation of diagenetically altered fats

A new approach to the identification of origin of remnant fats has been developed as part of this study based upon the greater resistance of certain triacylglycerol moieties to decay. The aim was to identify differences in the distributions of the more resilient triacylglycerol moieties in natural fats and oils, i.e. those which were more likely to survive in the archaeological record, using silver ion thin layer chromatography. Previous work by Breckenridge and Kuksis (1969) has shown the separation of triacylglycerol components in bovine milk fat using this procedure, enabling the distributions of components varying in degree of unsaturation to be determined.

In this chapter the distributions of triacylglycerols containing saturated and monounsaturated fatty acyl moieties in reference fats and olive oil have been described and

compared with intact triacylglycerols retrieved from ethnographic and archaeological residues. The aim was to determine the usefulness of this parameter in drawing distinctions between diagenetically altered fats. Distinctions according to degree of unsaturation or stereospecificity have not been made as part of this study. The effect of decay on triacylglycerol distributions in lamb adipose, ruminant milk fat and olive oil will be considered further in the laboratory decay experiments described in Chapter 7.

5.2 Triacylglycerol distributions in modern reference fats and oils

5.2.1 Fractionation of modern reference fats and olive oil by silver ion thin layer chromatography

Fractionation of triacylglycerol species in modern reference animal fats was carried out using silver ion thin layer chromatography (TLC), enabling the isolation of components comprised of saturated fatty acyl moieties from their mono- and polyunsaturated counterparts. Details of the analytical procedures followed are given in Section 9.1.8. An indication of the number of fractions visualised is shown in Figure 5.1. The R_f values for the different bands are given in Table 1, Appendix 5 (p. 379) for selected reference fats and olive oil.

The bands eluting furthest up the plate with an R_f value of 0.7 contained saturated triacylglycerol components. No triacylglycerols were separable above R_f 0.58 for the olive oil due to the highly unsaturated nature of this commodity. Bands were scraped from the plate and lipid components extracted as described in Section 9.1.8. The individual fractions were analysed by HTGC using the conditions described in Section 9.2.2. The partial HTGC profiles obtained for four upper most fractions from the reference fats and the uppermost fraction in olive oil, are shown in Figures 5.4 to 5.11.

The elution order of triacylglycerols by silver ion TLC has been reported by Gunstone and Padley (1965). One dienoic fatty acyl residue forms stronger complexes with silver ions than two monoenoic fatty acyl residues in the same molecule and one trienoic acid is equivalent to two dienoic acids. In addition to the number of double bonds, both the chain length and the position of the double bond in the fatty acyl moiety has an influence on

separation (Nikolova-Damyanova *et al.*, 1990). In general, *trans* fatty acids form weaker complexes with silver ions than *cis* acids. Thus, the band eluting highest up the plate is expected to be comparable to the distributions of triacylglycerols which would result following preferential decay of the unsaturated components.

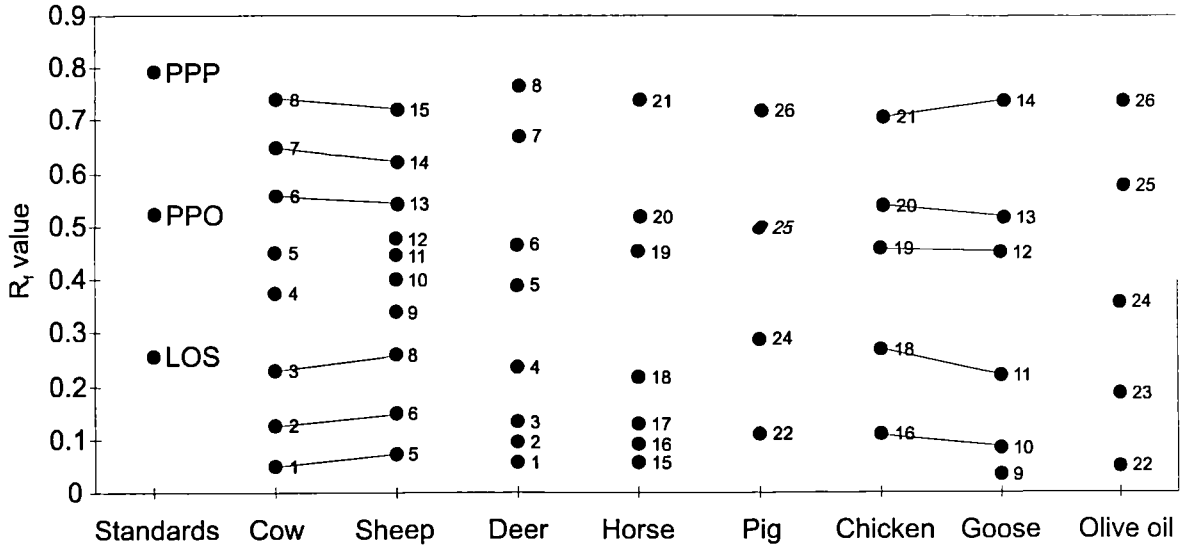


Figure 5.1 R_f values calculated for the triacylglycerols in reference animal fats following separation by silver ion TLC (20% plates) compared with pure triacylglycerol standards: PPP=1,2,3-palmitoyl glycerol; PPO=1(3),2-palmitoyl-3(1)-oleoyl glycerol; LOS=1(3)-linoleoyl-2-oleoyl-3(1)-stearoyl glycerol. The various bands relate to triacylglycerol components with differing degrees of unsaturation, thus the more highly unsaturated components have the lowest R_f values and *vice versa*. Three pure triacylglycerol standards are shown for comparison, illustrating that the sinurgic bonds formed with the unsaturated moieties in the LOS and PPO standards retards their travel up the plate compared with the saturated PPP component.

In Figure 5.1 the band eluting ca. R_f 0.65 in the ruminant fats alone corresponds to triacylglycerols containing one *trans*-configured mono-unsaturated isomer (i.e. fractions 7, 14 and 7, for the cow, sheep and deer fats, respectively). The band eluting ca. R_f 0.53 corresponds to unsaturated components with one *cis*-configured monounsaturated isomer, present in all of the reference fats and olive oil.

The position and number of spots eluting on the TLC plates are comparable for cow and sheep adipose, and chicken and goose fat; similarities can also be seen between the horse and deer adipose fats. There is a greater range of triacylglycerol moieties present in the ruminant compared with the non-ruminant fats, as can be seen by the number of fractions

separable by TLC. This reflects the greater variety of straight-chain, branched-chain and positional and geometric unsaturated moieties observed when ruminant fat is hydrolysed (Section 4.2). The non-ruminant fats and olive oil are compositionally much less complex.

The uppermost fractions separated by TLC have been quantified (Table 2, Appendix 5, p. 380) and the triacylglycerol distributions plotted in histograms in order to compare the fats from different animals. It could be postulated that the saturated triacylglycerol fraction is representative of a highly degraded archaeological fat, whereas better preserved fats may resemble a combination of the upper fractions since we are frequently able to recover mono-unsaturated fatty acids (albeit in relatively minor proportions) through hydrolysis of intact triacylglycerols in archaeological fats. However, we do not observe the polyunsaturated fatty acids which are abundant in fresh fats, due to their relatively high chemical reactivity (Killops and Killops, 1993). In order to provide comparable distributions of intact triacylglycerols to those which would result according to different extents of decay, distributions have been considered which would occur if triacylglycerols contained: (i) saturated moieties alone; (ii) saturated and mono-unsaturated *trans*-configured isomers (ruminant fats only), and (iii) saturated and mono-unsaturated *cis*- and *trans*-configured isomers (ruminant and non-ruminant fats). The second category has been considered since *trans*-isomers are preserved in preference to their *cis*-configured counterparts (see Section 4.1.3). Histograms comparing these distributions are shown in Figures 5.2 and 5.3. Relative abundances have been calculated based on the percentages of triacylglycerols of different degrees of unsaturation to account for the different proportions of saturated and unsaturated moieties in different fats.

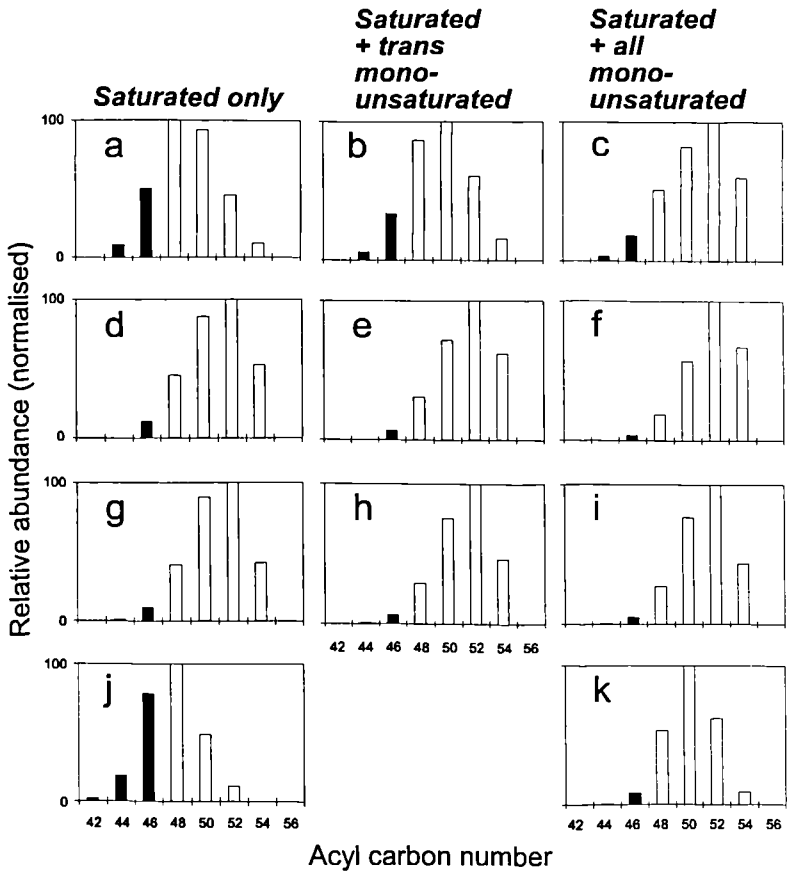


Figure 5.2 Distributions of intact triacylglycerols in modern reference animal fat fractions separated by silver ion TLC: (a) cow adipose, fr. 8/R_f 0.74; (b) cow adipose, sum of fractions 7 and 8; (c) cow adipose, sum of fractions 6, 7 and 8; (d) sheep adipose, fr. 15/R_f 0.72; (e) sheep adipose, sum of fractions 14 and 15; (f) sheep adipose, sum of fractions 13, 14 and 15; (g) deer adipose, fr. 8/R_f 0.76; (h) deer adipose, sum of fractions 7 and 8; (i) deer adipose, sum of fractions 6, 7 and 8; (j) horse adipose, fr. 21/R_f 0.74; (k) horse adipose, sum of fractions 20 and 21.

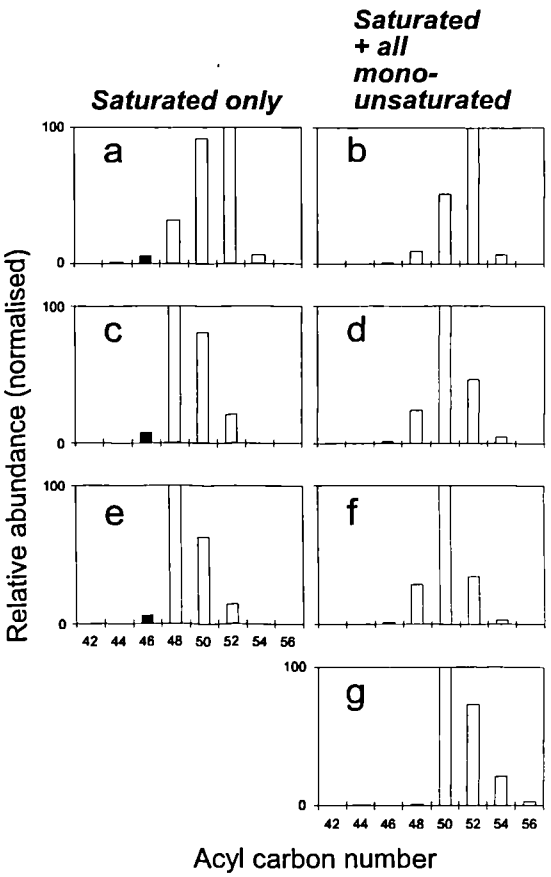


Figure 5.3 Distributions of intact triacylglycerols in the modern reference animal fat fractions separated by silver ion TLC: (a) pig fat, fr. 26/R_f 0.72; (b) pig fat, sum of fractions 25 and 26; (c) chicken fat, fr. 21/R_f 0.71; (d) chicken fat, sum of fractions 20 and 21; (e) goose fat, fr. 14/R_f 0.74; (f) goose fat, sum of fractions 13 and 14; (g) olive oil, fr. 25/R_f 0.58.

5.2.2 Bovine adipose

Bovine adipose contains saturated triacylglycerols ranging between C_{44} and C_{54} (Fig. 5.4). Notably, there is no saturated C_{52} present and the C_{44} and C_{54} components are minor. The predominant saturated triacylglycerol is C_{48}, with C_{50} also present in abundance. The overall distribution [Fig. 5.2 (a)] is not very different from horse adipose shown in Figure 5.2 (j), except for the presence of the C_{54} and the absence of the C_{42} component. The C_{50} component is approximately twice as abundant as the C_{52} in bovine adipose. The incorporation of components containing *trans*-configured moieties results in the increase in relative abundance of the C_{50} component [Fig. 5.2 (b)], and the addition of the *cis*-configured components results in the higher relative abundance of both the C_{52} and C_{54} components so that the C_{52} is predominant. Where the monounsaturated moieties are present, the C_{54} is also relatively high in abundance and the C_{48} relatively minor [Fig. 5.2

(c)]. Thus, in well-preserved archaeological bovine adipose fat it would be expected that the C_{52} to be predominant and not readily distinguishable from ovine adipose fat. In bovine adipose fat the *cis*-configured fraction makes up a significantly higher proportion of the fat than the *trans*-configured fraction, and notably, the C_{54} is predominant in the former which would account for the ease by which the C_{54} component is lost from archaeological bovine fats. Cow's milk fat was not analysed by TLC since the effect of decay on its triacylglycerol composition has been studied in detail in Chapter 7.

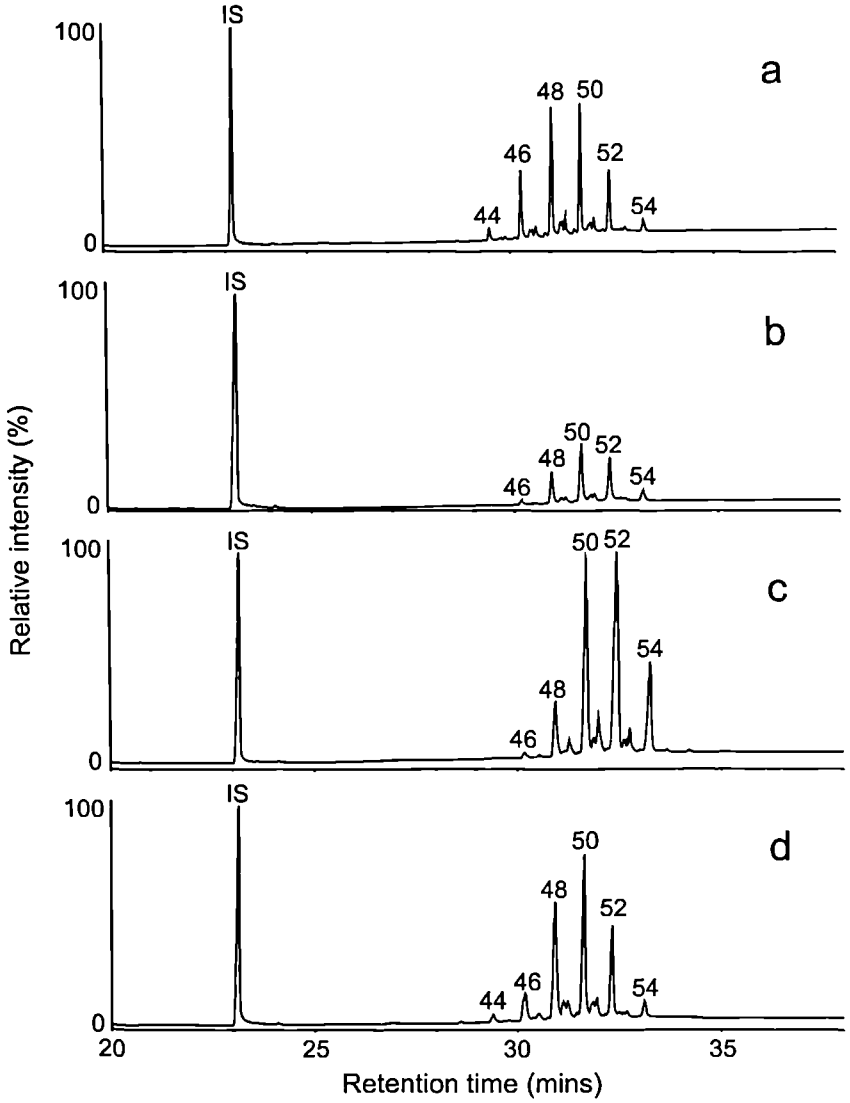


Figure 5.4 Partial HTGC profiles of the intact triacylglycerols in cow adipose fat: (a) fr. 8/R_f 0.74; (b) fr. 7/R_f 0.65; (c) fr. 6/R_f 0.56; (d) fr. 5/R_f 0.45. IS refers to internal standard (tricaprylin) added to each fraction following removal from the TLC plate; 44, 46 etc. refer to the total number of acyl carbon atoms on the glycerol backbone of the triacylglycerol. Chromatographic conditions are as described in Section 9.2.1.

5.2.3 Ovine adipose

The saturated fraction of ewe adipose fat is comprised of a distribution of triacylglycerols ranging between C_{46} and C_{54} [Fig. 5.2 (d) and Fig. 5.5]. The C_{54} is significantly more abundant than in the same fraction in the bovine adipose fat [Fig. 5.2 (a)]. The lack of the C_{44} component and the predominance of the C_{52} in ovine adipose may enable distinctions to be drawn between degraded fats from an ovine and bovine origin, particularly since the latter component is relatively minor in the more saturated triacylglycerol fractions of bovine adipose. Degraded ovine adipose would be expected to contain a relatively high abundance of C_{54} since high abundances of saturated and *trans*-monounsaturated C_{54} components are present in the fat, compared with, e.g. bovine adipose. Considering the fact that cows and sheep are both ruminant animals, the distributions of the saturated triacylglycerol moieties comprising their adipose fats are readily distinguishable.

The preservation of triacylglycerols containing *trans*-configured monounsaturated fatty acid moieties in archaeological fats would slightly increase the relative abundance of the C_{50} component [Fig. 5.2 (e)], although in general the distribution would not change significantly. Nor would the preservation of *cis*-configured isomers change the distribution noticeably [Fig. 5.2 (f)]. Thus, the C_{52} component would predominate in degraded fats, with the C_{54} in relatively high abundance.

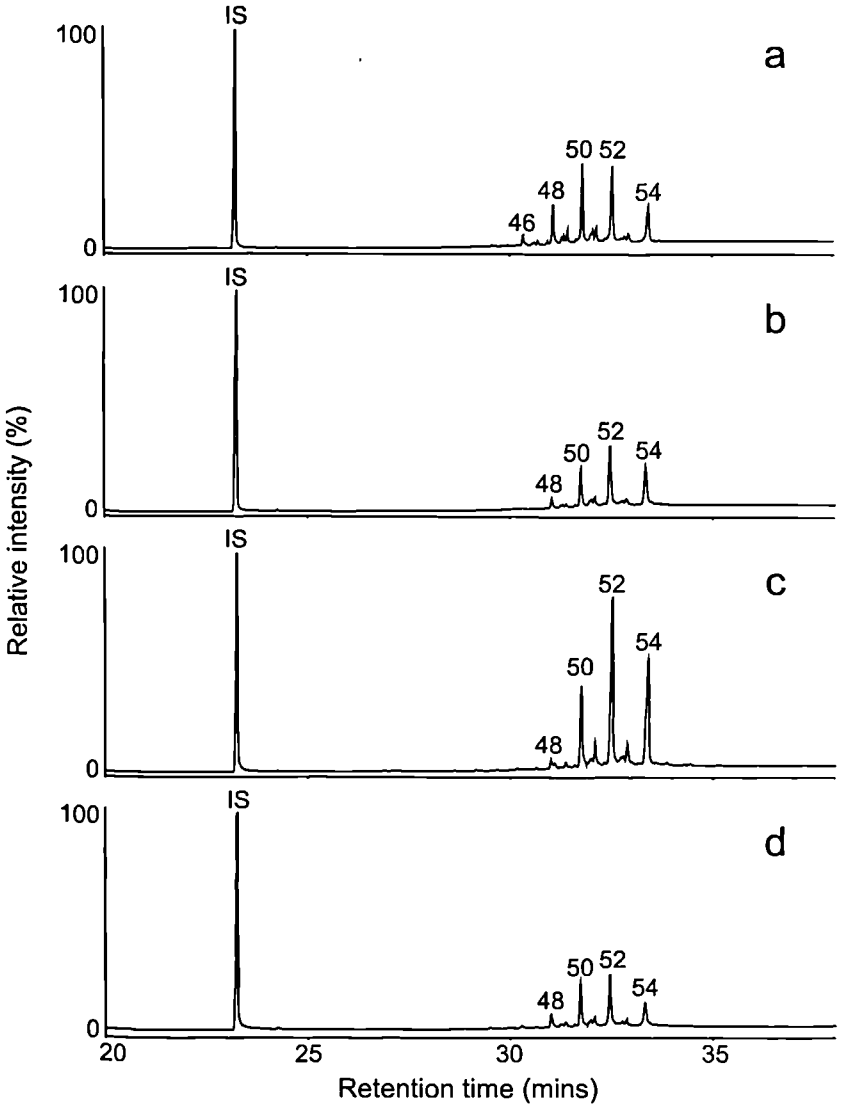


Figure 5.5 Partial HTGC profiles of the intact triacylglycerols in sheep adipose fat: (a) fr. 15/R_f 0.72; (b) fr. 14/R_f 0.62; (c) fr. 13/R_f 0.54; (d) fr. 12/R_f 0.48.

5.2.4 Cervine fats

The saturated triacylglycerol fraction in deer (cervine) adipose bears a close resemblance to ovine adipose, although the relative abundances of the C_{50} and C_{52} are higher in the deer adipose (Fig. 5.6). There is a relatively high abundance of saturated and mono-unsaturated *trans*-configured components comprising the C_{54} triacylglycerols, which would probably contribute to a relatively high abundance of intact C_{54} in decayed cervine fats, as in ovine fats. The distribution of triacylglycerols in the deer adipose is also similar to ovine adipose when considering the combined saturated and mono-unsaturated fractions, except that in

deer fat [Fig. 5.2 (i)] the proportions of the C_{48} and C_{50} components are slightly higher than in ovine adipose [Fig. 5.2 (f)].

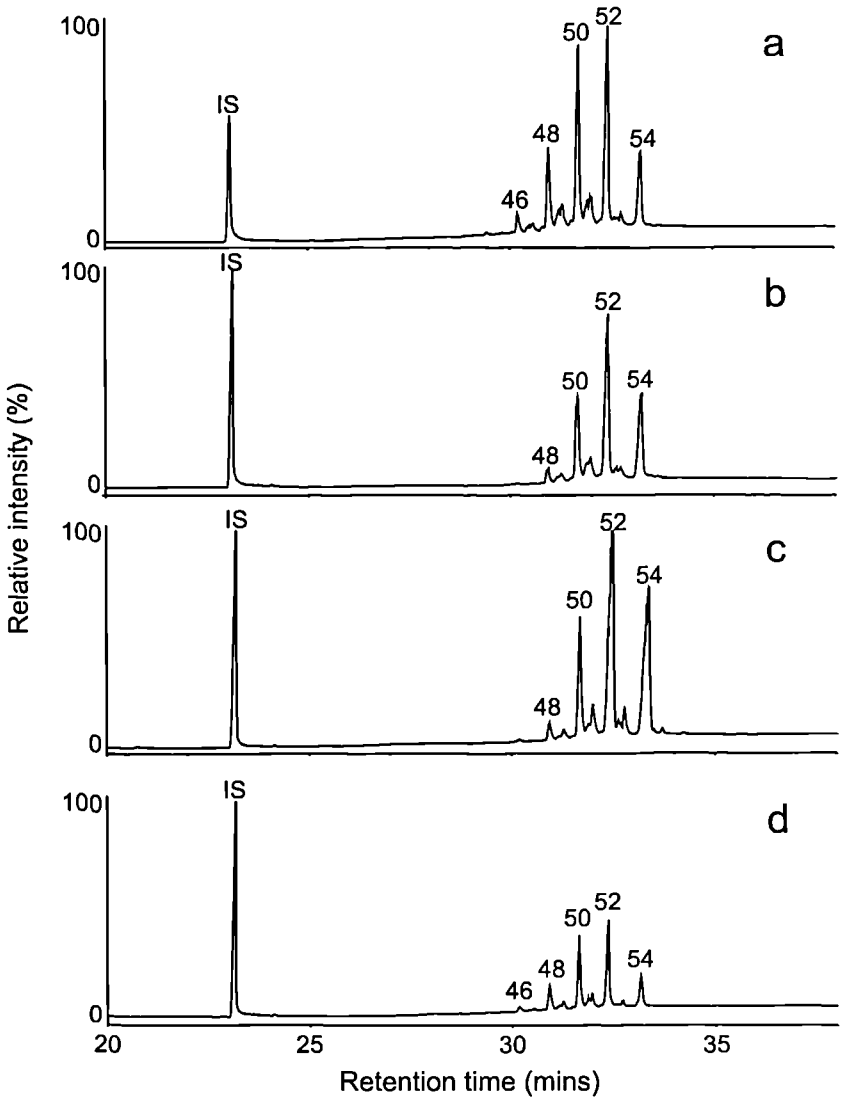


Figure 5.6 Partial HTGC profiles of the intact triacylglycerols in deer adipose fat: (a) fr. 8/R_f 0.76; (b) fr. 7/R_f 0.67; (c) fr. 6/R_f 0.46, and (d) fr. 5/R_f 0.39.

5.2.5 Equine fats

Although the R_f values and number of fractions separated by TLC are comparable for the horse and deer fats, there is no similarity between the distributions of individual triacylglycerol moieties separated by HTGC from these two fat types (Fig. 5.7). The saturated triacylglycerol fraction of the horse fat is clearly distinguishable from ruminant adipose fats on account of the complete lack of the C_{54} component. The characteristically low abundance of the $C_{18:0}$ fatty acid in horse fat is reflected in the lower abundance of

higher molecular weight triacylglycerol components. The majority of C_{52} is comprised of $C_{18:1}$ and therefore highly decayed horse adipose would be expected to contain a low abundance of both C_{52} and C_{54}. In the saturated fraction the C_{46} and C_{48} triacylglycerols dominate with relatively minor abundances of the C_{42}, C_{44} and C_{52} components.

The incorporation of compounds containing *cis*-configured monounsaturated fatty acyl moieties transforms the distribution, with higher relative abundances of the C_{50}, C_{52} and C_{54} components. Thus in an archaeological equine adipose fat in which unsaturated components are preserved, the C_{50} is likely to predominate, with the C_{46}, C_{48} and C_{54} in relatively low abundance [Fig. 5.2 (k)].

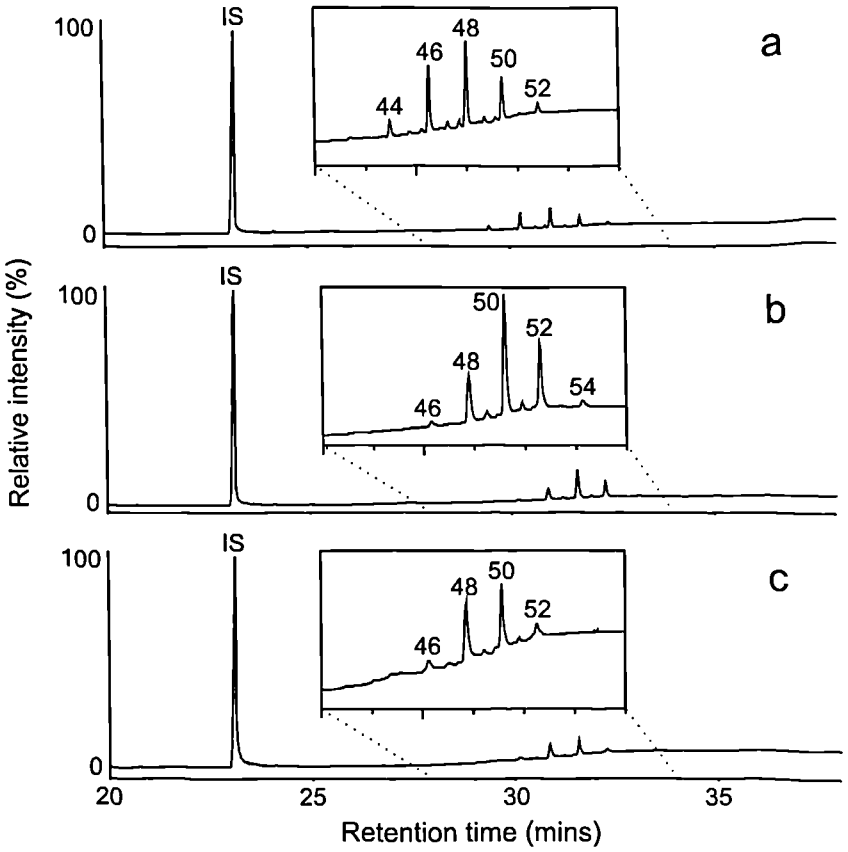


Figure 5.7 Partial HTGC profiles of the intact triacylglycerols in horse adipose fat: (a) fr. 21/R_f 0.74; (b) fr. 20/R_f 0.52; (c) fr. 19/R_f 0.45.

5.2.6 Porcine fats

Saturated triacylglycerols in porcine fats range between C_{44} and C_{54}, with the C_{52} component dominant and the C_{50} in slightly lower abundance (Fig. 5.8).

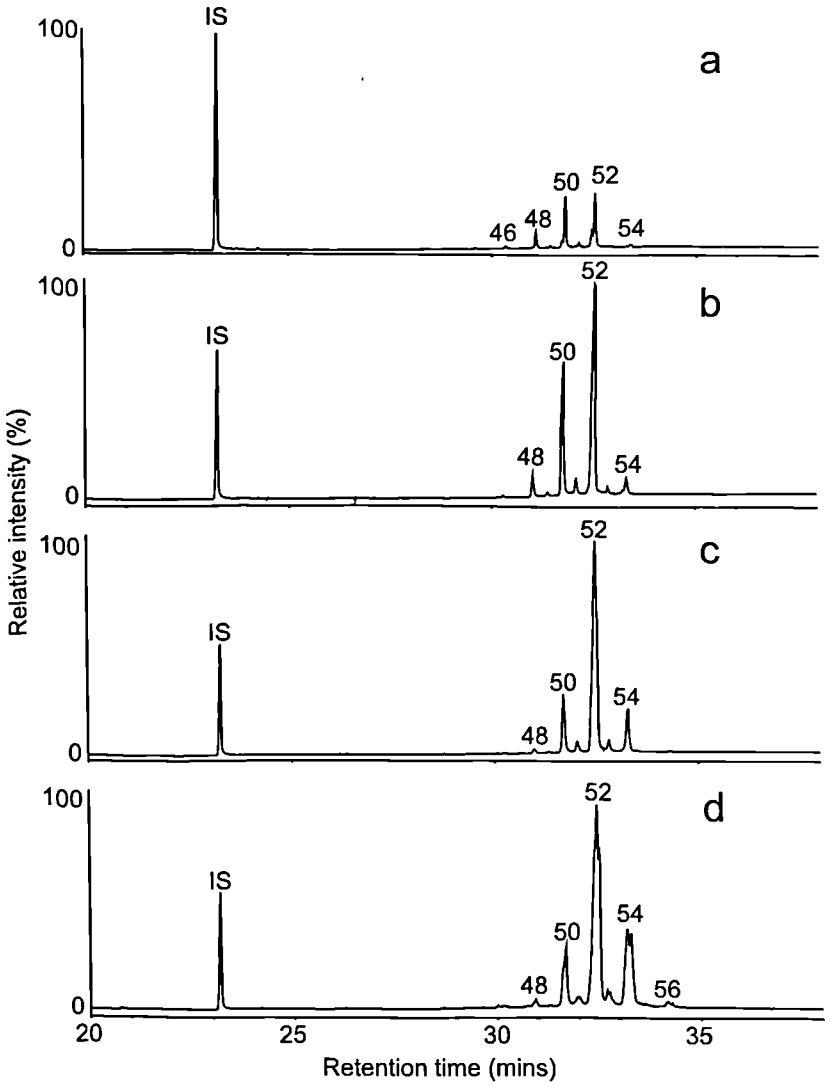


Figure 5.8 Partial HTGC profiles of the intact triacylglycerols in porcine adipose fat: (a) fr. 26/R_f 0.72; (b) fr. 25/R_f 0.5; (c) fr. 24/R_f 0.29; (d) fr. 22/R_f 0.11.

In comparison, the C_{46}, C_{48} and C_{54} are minor components. Porcine fats are thus quite distinctive compared with the other major domesticates, namely ovine and bovine species due to the clear dominance of the C_{50} and C_{52} over all of the other saturated components which gives a characteristic 'narrow' distribution. The difference in abundance between the C_{52} and C_{54} components and the lack of the C_{44} are also notable. The majority of the C_{54} component and all of the C_{56} in pig fat are comprised of at least one unsaturated fatty acid moiety, probably resulting in their very low abundance in degraded porcine fats. The saturated fraction of porcine adipose comprises a relatively small proportion of the total fat, as shown in Figure 5.8 where the different fractions are compared with the internal standard. Figure 5.3 (a) and (b) show the change to the profile if the triacylglycerols

containing *cis*-configured moieties are incorporated, whereby there would be a significantly higher proportion of the C_{52} at the expense of the C_{50}, but still relatively low proportions of the C_{46}, C_{48} and C_{54} components.

5.2.7 Poultry

5.2.7.1 Chicken fat

Chicken fat contains saturated triacylglycerols in the range C_{46} to C_{52}, with C_{48} the most abundant component (Fig. 5.9). The C_{46} and C_{52} are both relatively minor in abundance. In bovine and equine adipose the C_{48} component is also predominant, however, in these fats there are significantly higher abundances of the C_{44} and C_{46} triacylglycerols. The C_{52} and C_{54} components are mostly comprised of unsaturated moieties, likely to lead to their low abundance in archaeological fats. In combination, the saturated and monounsaturated fractions shown in Figure 5.3 (d) contain the C_{50} as the predominant component with less abundant C_{48} and C_{52} and minor C_{54}. The saturated components make up only a small proportion of the chicken fat shown by comparison with the abundance of lipid extracted from the other TLC fractions and quantified using the internal standard. The addition of triacylglycerols containing *cis*-configured monounsaturated moieties may result in a profile similar to that shown in Figure 5.3 (d), with a higher abundance of the C_{50} and C_{52} relative to the C_{48} than seen in the saturated fraction alone, and a predominance of the C_{50}. There is a low abundance of the C_{54} component in both the saturated and monounsaturated fractions of the poultry fats.

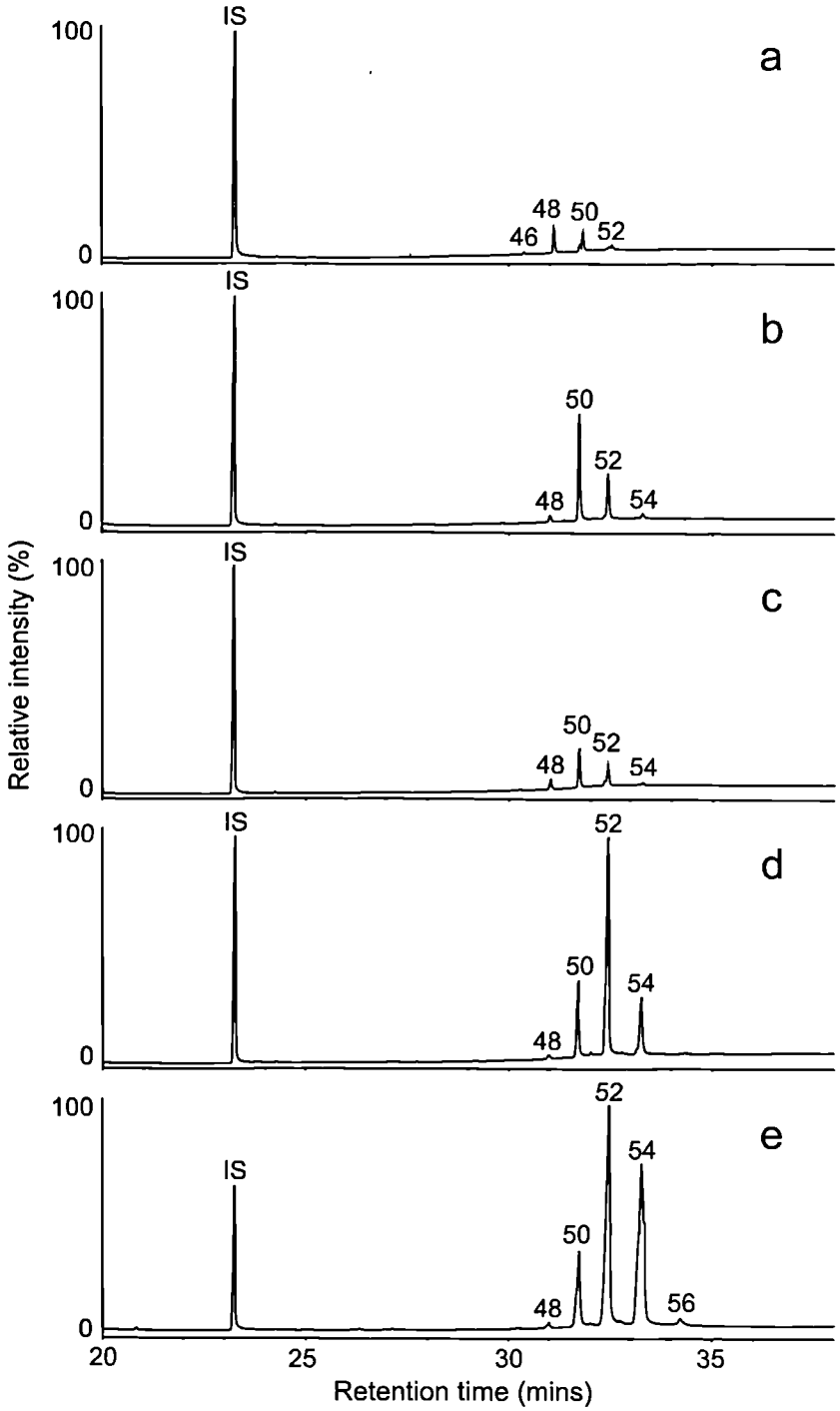


Figure 5.9 Partial HTGC profiles of the intact triacylglycerols in chicken fat: (a) fr. 21/R_f 0.71; (b) fr. 20/R_f 0.54; (c) fr. 19/R_f 0.46; (d) fr. 18/R_f 0.27.

5.2.7.2 Goose fat

The range and relative abundances of triacylglycerols in goose fat are almost identical to the distribution seen in chicken fat, with the saturated fraction characterised by abundant C_{48} and C_{50} with minor C_{46} and C_{52} components (Fig. 5.10). No saturated C_{54} component is

present in goose fat. The incorporation of mono-unsaturated triacylglycerols would yield a similar distribution to that of chicken fat [Fig. 5.3 (f)].

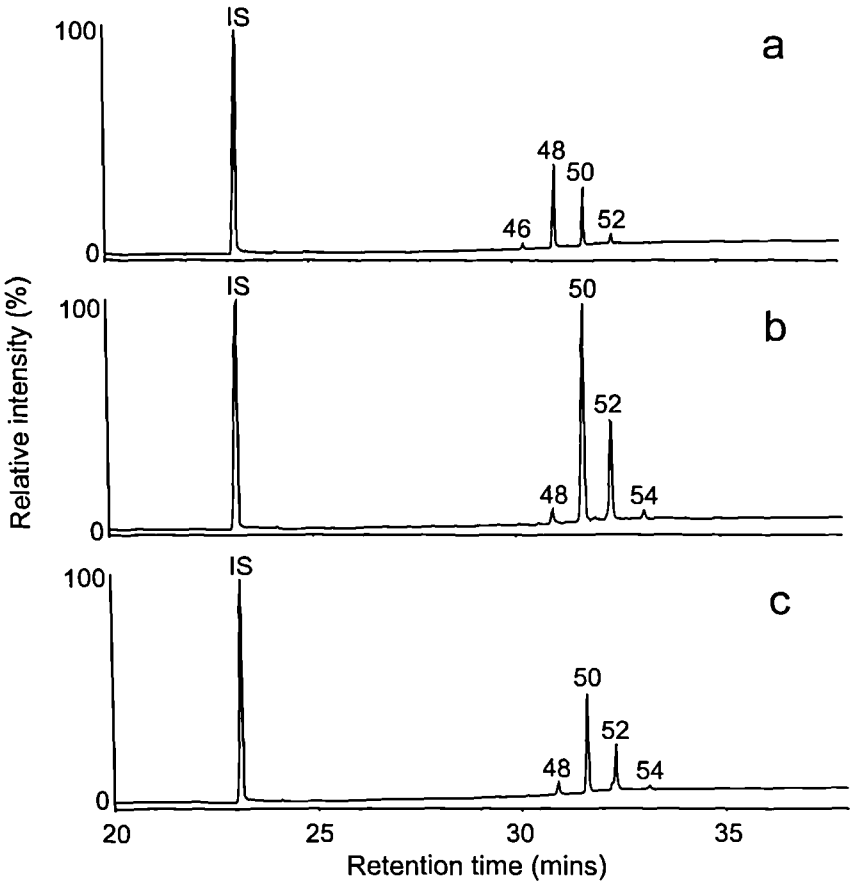


Figure 5.10 Partial HTGC profiles of the intact triacylglycerols in goose fat: (a) fr. 14/R_f 0.74; (b) fr. 13/R_f 0.52; (c) fr. 12/R_f 0.45.

5.2.8 Olive oil

The uppermost fraction visualised on the TLC plate eluted at R_f 0.74, although the abundance of lipid was so small that no peaks were detectable by HTGC analysis. The fraction eluting at R_f 0.58 is shown in Figure 5.3 (g) and is comprised predominantly of C_{50}, C_{52} and C_{54} triacylglycerols, with relatively minor C_{48} and C_{56}. The most abundant component is the C_{50} triacylglycerol (Fig. 5.11).

Olive oil is distinguishable from chicken and goose fat by the significantly lower abundance of the C_{48} component than in the poultry fats. The distribution of total triacylglycerols in fresh olive oil is shown in Figure 5.12 (a). The intact oil contains a predominance of the C_{52} component and the profile is completely different from the

distribution of monounsaturated triacylglycerols separated by TLC [no completely saturated triacylglycerols were separable; Fig. 5.12 (b)].

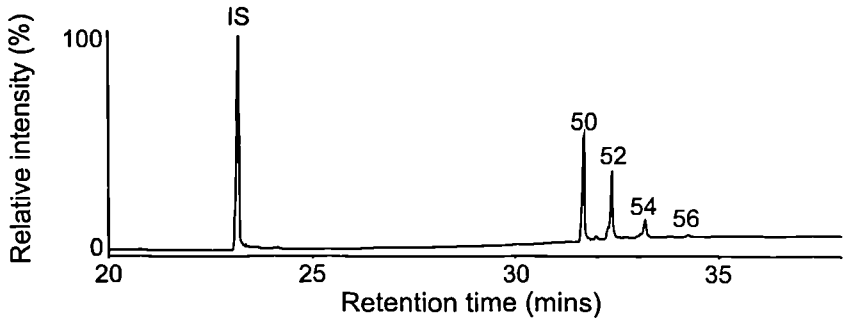


Figure 5.11 Partial HTGC profiles of the intact triacylglycerols in olive oil, fr. 25/R_f 0.58.

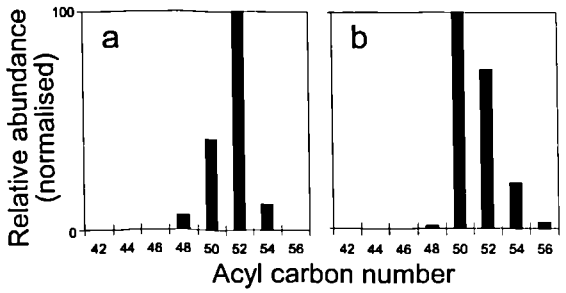


Figure 5.12 Distributions of triacylglycerols in (a) whole fresh olive oil, and (b) olive oil TLC fraction 25/R_f 0.58.

5.2.9 Diagnostic distributional characteristics of triacylglycerols in reference fats and olive oil

In order to make comparisons between the different archaeological fats and between the archaeological and modern reference fats, consideration has been given to a number of diagnostic characteristics noted in the distributions of triacylglycerols in the saturated fractions of the modern reference fats shown in histograms in Figures 5.2 and 5.3. The criteria considered most useful in drawing distinctions include the following:

I Maximises at C_{52}

II C_{52} less than 75% of C_{50}

III Maximises at C_{48} or C_{50}

IV Maximises at C_{50} (with C_{48} <50% of C_{50})

V Approximately equal abundance of C_{50} and C_{54} (both <75% of C_{52})

VI C_{54} <0.33% of most abundant component

VII High abundance of $C_{54} \geq 50\%$ of most abundant component

VIII Presence of C_{44}

IX C_{48} and C_{54} <50% of most abundant component

X Presence of C_{40} and/or C_{42} and above

XI Presence of C_{56}

XII Maximises at C_{48}

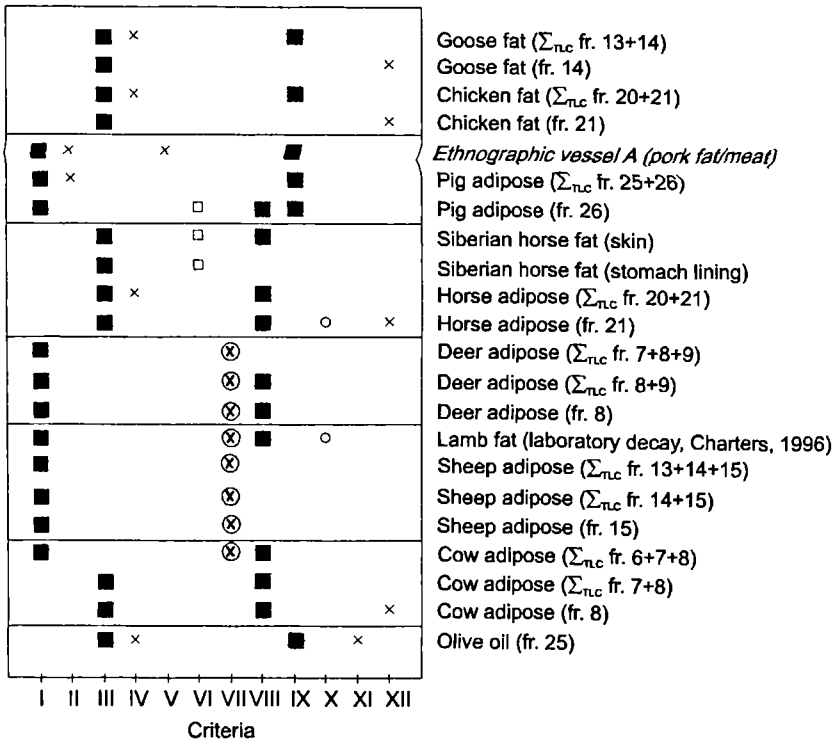


Figure 5.13 Characteristics of intact triacylglycerol distributions in reference fats. The characteristics of the Siberian horses, lamb fat from decay experiments and pork fat from the ethnographic vessel. The various criteria used are described in the text.

The specific characteristics which apply to the different reference fats are shown in Figure 5.13. Several criteria in particular have proven most useful in drawing distinctions between the different fats. Porcine, ovine and cervine fats are separable based on criterion **I**, comprising a distribution which maximises at C_{52}, and cervine and ovine fats are further distinguished by criterion **VII**, on account of their comprising a relatively high abundance of C_{54}; a low abundance of C_{54} (<0.33% of the most abundant triacylglycerol; criterion **VI**) rules out the possibility that a fat derives from an ovine or a cervine source. Porcine fats are separable from ovine and cervine fats based upon criterion **IX**, which means they contain very low relative abundances of both the C_{48} and C_{54} components. Bovines, poultry

and equines are characterised by criterion **III**, with their triacylglycerol distributions maximising at C_{50}. Equine fats and dairy fats alone contain lower-carbon number triacylglycerols starting at C_{40} or C_{42} (acyl carbon number; criterion **X**). These indicators of animal origin have been applied to the triacylglycerol distributions preserved in remnant archaeological fats and the results are discussed in Section 5.3.

5.3 Triacylglycerol distributions in archaeological and ethnographic fats and oils

5.3.1 Sites with well-documented faunal assemblages

5.3.1.1 West Cotton (Late Saxon/early medieval)

Intact triacylglycerols were present in all of the total lipid extracts selected for analysis from the West cotton assemblage; WC30 has also been included for comparison (Fig. 5.14; Table 3, Appendix 5, pp. 380-381). In general the distributions ranged between C_{40} and C_{54}; no C_{56} components were detected in the extracts. Based upon a combination of distributional criteria (Fig. 5.15) tentative distinctions have been made between animals believed to have been the major domesticated species at West Cotton during the Late Saxon/early medieval period. The data indicate that the majority of remnant fats from West Cotton derive either from a porcine, ovine or dairy origin, or possibly represent a mixture of different fats. Since we know from the faunal remains that no goats were present at West Cotton, fats identified as caprine can be defined as sheep fats.

None of the triacylglycerol distributions from the West Cotton vessels correlated closely with those observed in the reference goose and chicken fats. The distribution in the majority (*ca* 55%) of extracts resembled sheep adipose fat based upon the relative abundance of the C_{52} and C_{54} components and a relatively narrow distribution of components overall. None of the distributions matched that of the reference bovine adipose on the basis of the C_{50} being the most abundant component, although it is possible that diagenetic alteration has modified archaeological bovine fats so that they resemble ovine adipose fats (see Section 6.7 for discussion). Bovine fats may be distinguishable on the basis of the presence of the C_{44} component and a relatively low abundance of the C_{54}, characteristics which indicate that RP13 resembles degraded bovine fat. RP28 and RP50 have been assigned as mixtures, possibly of sheep and pig adipose since they comprise

relatively narrow distributions, with C_{52} predominating and relatively low abundances of C_{54}.

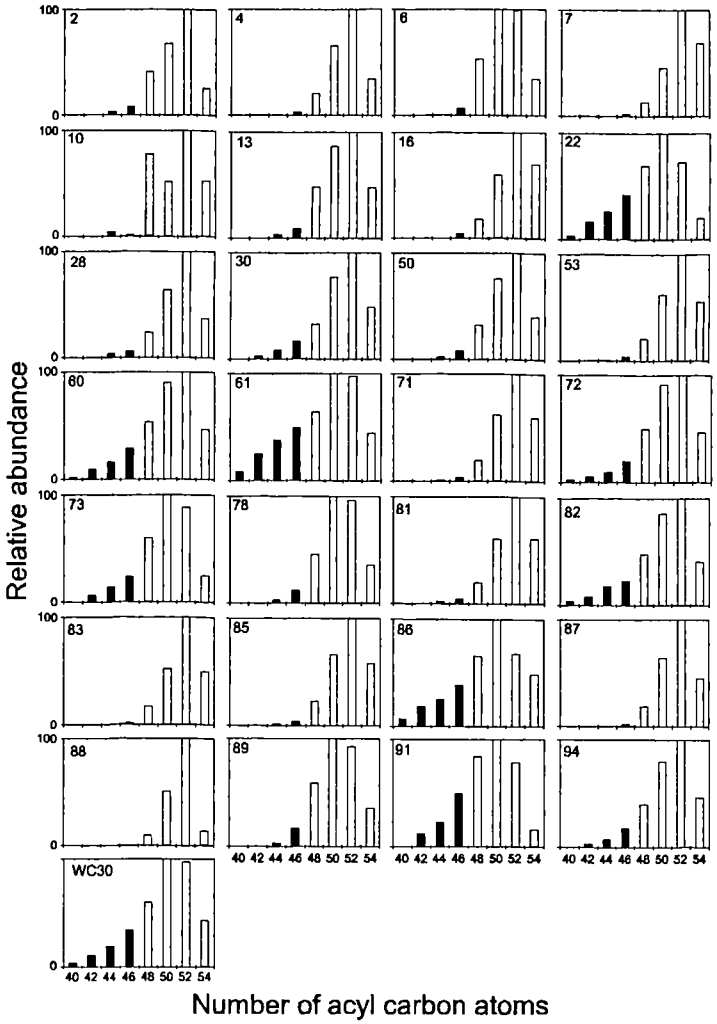


Figure 5.14 Histograms showing the carbon number distributions of triacylglycerols (acyl carbon number) in total lipid extracts of potsherds from the Late Saxon/early medieval vessels recovered from excavations at West Cotton. The relative abundance of triacylglycerols was calculated by measuring the peak area in the HTGC profile.

Dairy fats appear to be well represented amongst the West Cotton vessels based upon the presence of lower carbon-number triacylglycerols, such as C_{40} and C_{42}, which contribute to a much more broad and distinctly characteristic range of components. The lower carbon-number triacylglycerols are present in RP22, 60, 61, 86, 91 and WC30, and are known to be highly abundant in fresh dairy fats. The distributions of triacylglycerols in diagenetically altered dairy fats is discussed further in Chapter 7. Figure 5.15 shows that 10 sheep adipose, 2 deer (or sheep) adipose, 3 pig adipose, 11 dairy fats and 3 mixtures of different fats have been tentatively identified at West Cotton. The dairy fats have been

classified as deriving either from an ovine or bovine origin based upon the relative abundances of the C_{50} and C_{52} components, where ovine dairy fats have a higher abundance of the latter component and are also characterised by a relatively high abundance of the C_{54} component. RP10 has a relatively narrow distribution of intact triacylglycerols characteristic of non-ruminant fats but the abundance of the C_{54} component indicates that it is derived from sheep adipose. There appears to be some co-elution evidenced by the high abundance of C_{48} which indicates a mixture of different fats.

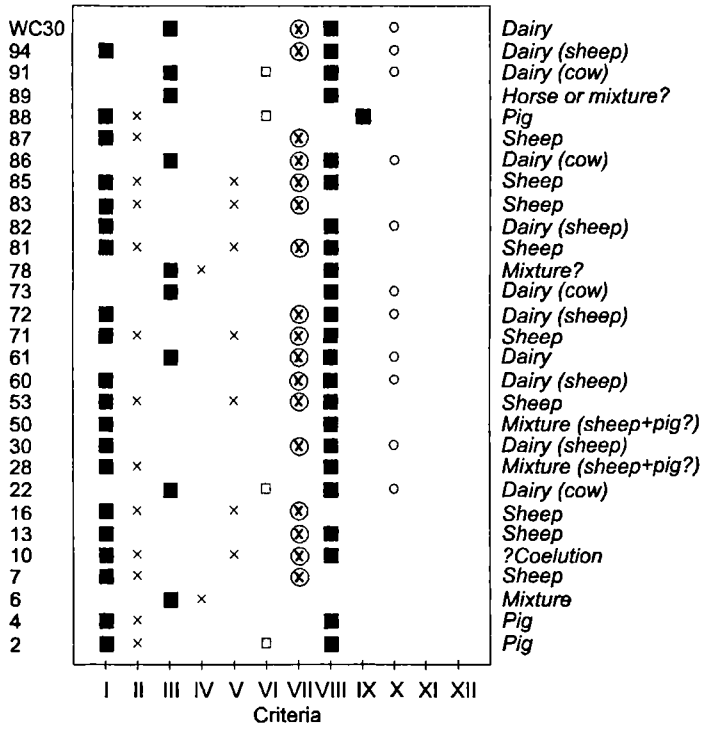


Figure 5.15 Characteristics of intact triacylglycerol distributions in archaeological fats from West Cotton and suggested origins for the remnant fats based upon comparisons with triacylglycerol distributions in reference fats.

5.3.1.2 Stanwick (Iron Age/Romano-British)

Intact triacylglycerols were present in all of the selected Stanwick extracts and their distributions are shown in Figure 5.16 (Table 4, Appendix 5, p. 381). Distributions ranged between C_{40}, C_{42} or C_{46} to C_{54}, and ST194 also contained a low abundance of the C_{38} component.

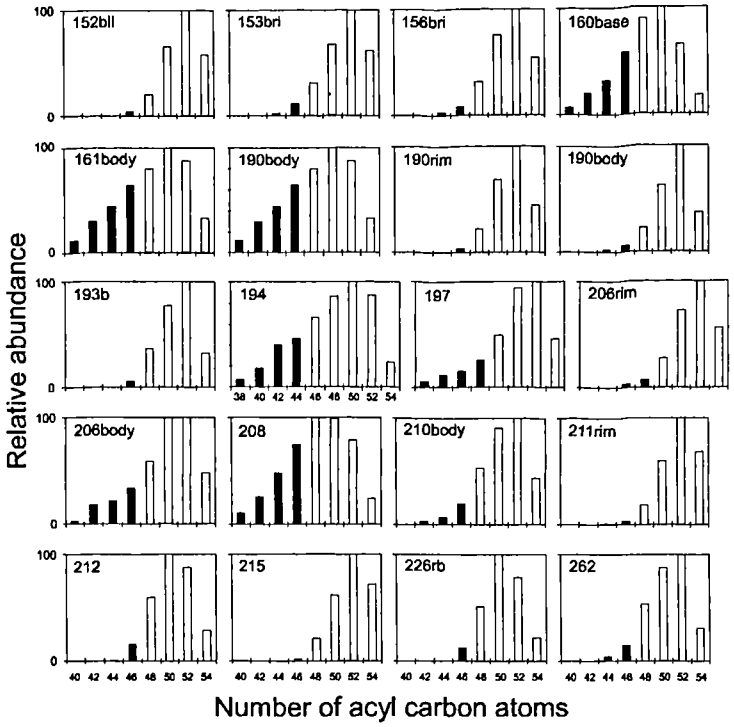


Figure 5.16 Histograms showing the carbon number distributions of triacylglycerols (acyl carbon number) in total lipid extracts of potsherds from the Iron Age/Romano-British vessels recovered from excavations at Stanwick.

Assignments made upon the basis of the distributional criteria are shown in Figure 5.17. Due to the higher relative abundance of the C_{52} component and the relatively broad distribution of triacylglycerols, ST262 and ST190 body have been assigned as mixtures of different fats, possibly ovine and porcine fats, since the C_{54} is in lower abundance than would be expected for ovine fats. A relatively high abundance of the C_{50} components, low C_{54} and a relatively broad overall distribution indicate that samples ST226rb and ST212 derive from bovine adipose. As at West Cotton, the majority of the extracts appear to derive from ovine animals; seven extracts (64% of fats designated an adipose origin) have been identified as sheep adipose on account of the relatively high abundance of C_{54} and the predominance of the C_{52} component. The remainder have been classed as dairy fats, primarily due to the presence of the lower carbon-number triacylglycerols (C_{40} and C_{42}). Dairy fats have been distinguished as either having an ovine or bovine origin based upon the relative abundances of the C_{50} and C_{52} components, with a 50% higher frequency of dairy fats originating from a bovine source. Thus on the basis of intact triacylglycerol distribution, the majority of the remnant adipose fats from both the Stanwick and West Cotton assemblages have been identified as ovine, but at Stanwick a larger proportion of

dairy fats (35% of the total) have been identified. No porcine fats have been identified at Stanwick based upon triacylglycerol distributions, although extracts from ST193b, ST262 and ST190 body have been tentatively identified as mixtures of ovine and porcine fats.

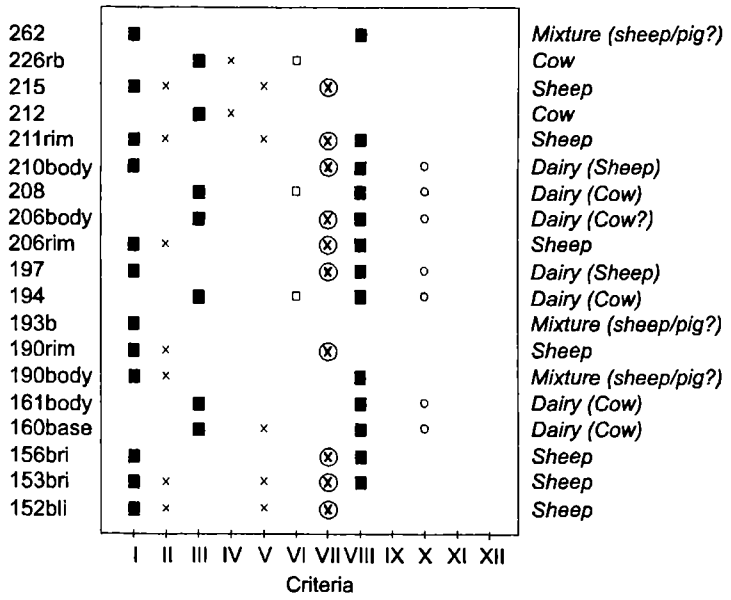


Figure 5.17 Characteristics of intact triacylglycerol distributions in archaeological fats from Stanwick and suggested origins for the remnant fats based upon comparisons with triacylglycerol distributions in reference fats.

5.3.2 Sites with an unusually strong bias in the faunal record

5.3.2.1 Wickham Bonhunt (Romano-British/Middle Saxon)

All of the samples yielding lipid extracts (14 out of a total of 19) from Wicken Bonhunt contained intact triacylglycerols. The distributions of components were similar in all extracts (Fig. 5.18; Table 5, Appendix 5, p. 382). The ranges were all between C_{44} or C_{46} to C_{54}, so none of the extracts contained the lower carbon-number components seen at West Cotton and Stanwick, and all the distributions maximised at C_{52}. The range of different fats represented would therefore appear to be the most limited of all the sites studied so far.

The range of criteria considered in Figure 5.19 indicate that almost all of the remnant fats derive either from ovine or porcine adipose, with the majority more closely representative of ovine fats. Only one of the assignments (sample 6) proved inconclusive due to the relatively high abundance of the C_{50} component which predominates in cow adipose fats,

and a high abundance of C_{54} more closely associated with ovine fat. Sample 6 may represent a mixture of different fats. No dairy fats were identified in the Wicken Bonhunt pottery and no conclusive evidence has been found for the exploitation of cattle at this site.

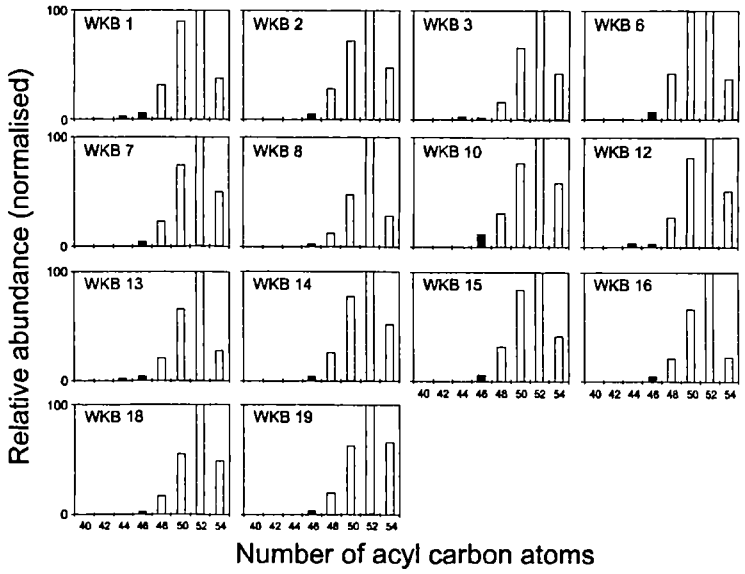


Figure 5.18 Histograms showing the carbon number distributions of triacylglycerols (acyl carbon number) in total lipid extracts of potsherds from the Romano-British/Middle Saxon vessels recovered from excavations at Wicken Bonhunt.

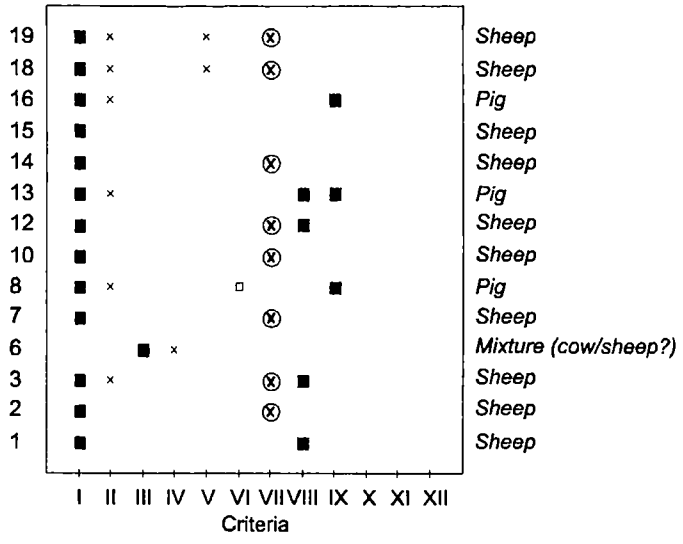


Figure 5.19 Characteristics of intact triacylglycerol distributions in archaeological fats from Wicken Bonhunt and suggested origins for the remnant fats based upon comparisons with triacylglycerol distributions in reference fats.

5.3.2.2 Fuller's Hill (Late Saxon/early medieval)

Trace abundances of intact triacylglycerols were identified in two of the extracts from Fuller's Hill, however they were only present in very low abundance and therefore difficult to quantify with accuracy.

5.3.2.3 Botai (early Neolithic)

Two of the 6 extracts from Botai which yielded lipid residues contained intact triacylglycerols (Fig. 5.20; Table 6, Appendix 5, p. 382). These ranged between C_{46} to C_{54} in sample N26 and C_{48} to C_{54} in sample XVIII. Neither extract contained the lower carbon-number (C_{40} and C_{42}) components characteristic of dairy fats, however differences between the distributions of triacylglycerols (Fig. 5.21) indicate different sources for these two remnant fats.

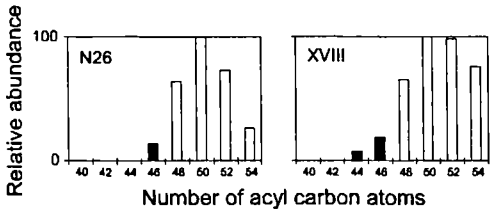


Figure 5.20 Histograms showing the carbon number distributions of triacylglycerols (acyl carbon number) in total lipid extracts of Early Neolithic potsherds recovered from excavations at Botai.

The relative abundances of C_{48} and C_{50} in sample N26 and the low abundance of the C_{54} are characteristic of a degraded equine adipose fat in which a proportion of monounsaturated components have been preserved [as seen in Fig. 5.2 (k)]. This assignment is supported by the long history of horse breeding associated with the site from which these vessels are derived.

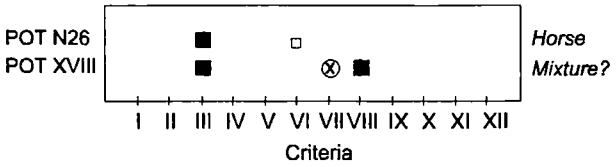


Figure 5.21 Characteristics of intact triacylglycerol distributions in archaeological fats from Botai and suggested origins for the remnant fats based upon comparisons with triacylglycerol distributions in reference fats.

The distribution seen in N26 is comparable with the reference horse fat [Fig. 5.2(k)], however, differs from the extracts of the Siberian horse fats (Fig. 5.33) mainly due to a lower abundance of the C_{48} component.

The distribution seen in the sample XVIII from Botai is less similar to the modern reference equine fats and more characteristic of a ruminant fat. This extract is distinguished from N26 by a high abundance of the C_{54} component. The distribution may represent a well-preserved fat derived from bovine adipose [Fig. 5.2 (c)] or a mixture of fats, since the C_{50} is present in higher abundance than would be expected in ovine adipose. It is notable, however, that this extract is not dissimilar from the muscle sample from the Siberian horse shown in Figure 5.33 (c).

5.3.3 Prehistoric archaeological sites

5.3.3.1 Yarnton Cresswell field (early-middle Iron Age)

Triacylglycerols were quantified from 20 out of 28 of the Yarnton Cresswell field extracts, yielding well preserved distributions which comprised a range of components including the more labile lower carbon-number moieties (Fig. 5.22; Table 7, Appendix 5, pp. 382-383).

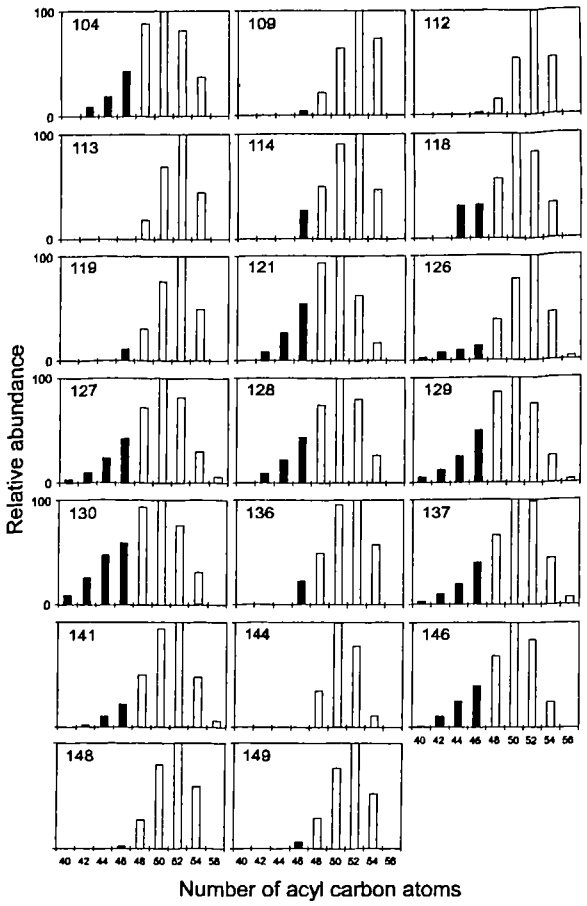


Figure 5.22 Histograms showing the carbon number distributions of triacylglycerols (acyl carbon number) in total lipid extracts of potsherds from the Early-Middle Iron Age vessels recovered from excavations at Yarnton Cresswell field.

Consideration of the various distributional criteria (Fig. 5.23) has indicated that 8 extracts derive from ovine adipose, and thus with one exception all of the adipose fats appear to derive from the same source. The relatively narrow range of components in sample 144 and the low abundance of the C_{46} component indicates a non-ruminant source or perhaps a mixture of fats. The remaining 11 extracts have been identified as dairy fats of both ovine and bovine origin.

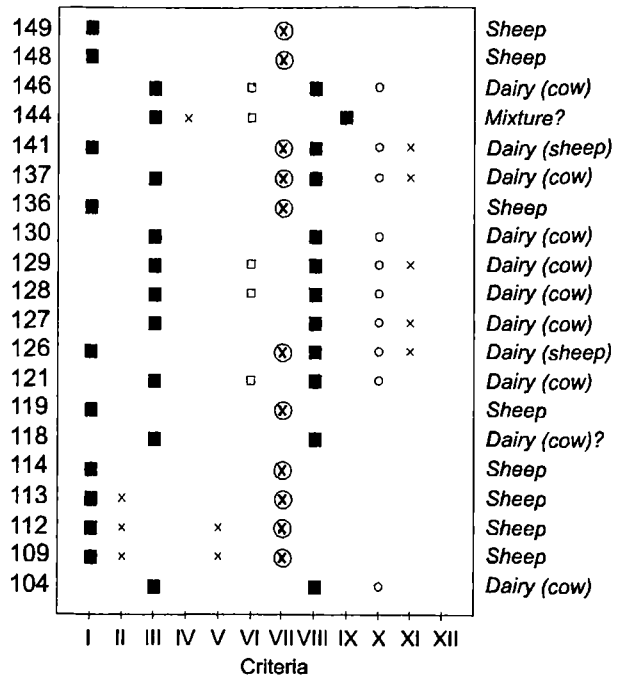


Figure 5.23 Characteristics of intact triacylglycerol distributions in archaeological fats from Yarnton Cresswell field and suggested origins for the remnant fats based upon comparisons with triacylglycerol distributions in reference fats.

5.3.3.2 Yarnton flood plain (Neolithic-Bronze Age)

Intact triacylglycerols were detected in 14 out of 25 Yarnton flood plain extracts, with high abundances of intact triacylglycerols indicating the excellent preservation afforded to these prehistoric fats (Fig. 5.24; Table 8, Appendix 5, p. 383). Eight of the extracts have been identified as dairy fats, from both bovine and ovine sources, and the extract from sample 38 is clearly characteristic of a porcine fat (Fig. 5.25). Samples 43 and 49 are considered to represent ovine adipose and the remainder are possibly mixtures of different fats or represent well-preserved bovine adipose, comparable with the distribution shown in Figure 5.2 (c). Extracts from samples 44 and 50 possibly represent mixtures. Due to the very low abundance of intact triacylglycerols in sample 40, only the C_{48} to C_{54} components could be reliably quantified, however, where other extracts from Yarnton flood plain constitute a comparably high abundance of the C_{48} component, there are also lower-carbon number components present. Thus it is possible that the extract from sample 40 also represents a dairy fat.

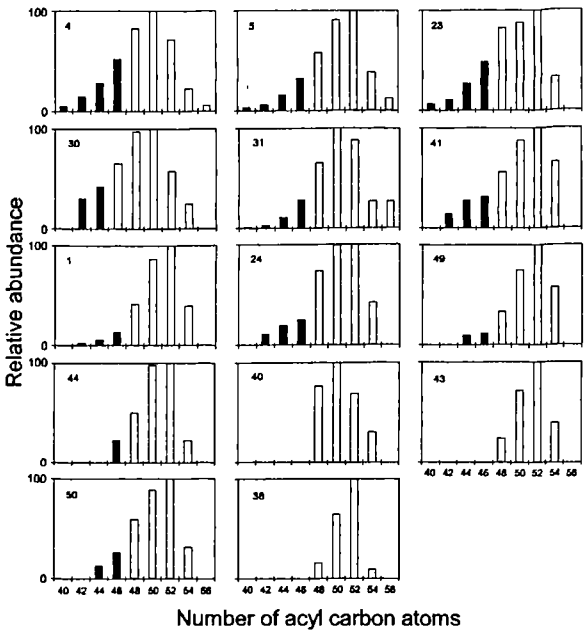


Figure 5.24 Histograms showing the carbon number distributions of triacylglycerols (acyl carbon number) in total lipid extracts of Neolithic potsherds recovered from excavations at Yarnton flood plain.

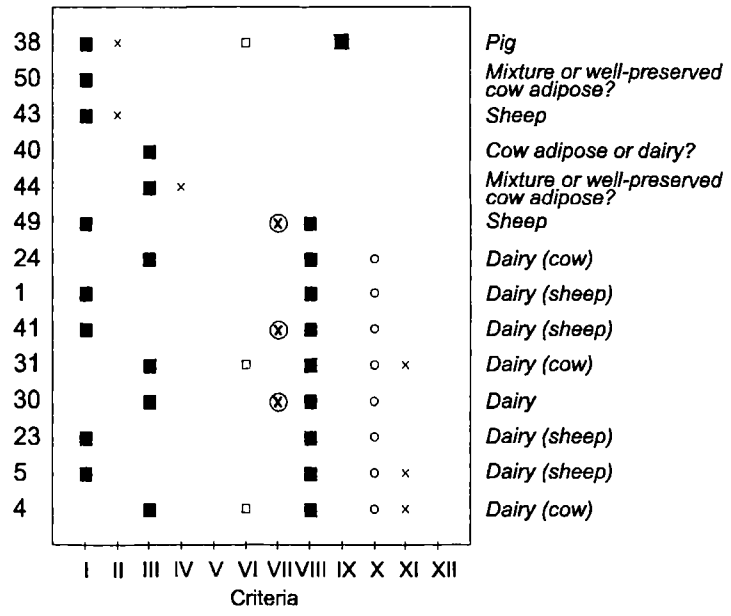


Figure 5.25 Characteristics of intact triacylglycerol distributions in archaeological fats from Yarnton flood plain and suggested origins for the remnant fats based upon comparisons with triacylglycerol distributions in reference fats.

5.3.3.3 Eton Lake End Road (late Neolithic-Early Bronze Age)

Twelve of the 14 extracts from Eton Lake End Road which yielded lipid residues contained intact triacylglycerols (Fig. 5.26; Table 9, Appendix 5, p. 384). The distributions

exhibit a range of characteristics, including both broad and narrow ranges indicating the presence of both dairy and adipose fats (Fig. 5.27). Consideration of the different characteristics indicate that 7 extracts out of 12 derive from dairy fats, probably all from a bovine source. Two further extracts, from samples NRA5 and NRA3 possibly also represent dairy fats, although the triacylglycerols were only present in very low abundance. An unidentified component, possibly a phthalate, co-eluting with the C_{44} triacylglycerol was present in samples NRA3, NRA5 and NRA10 which made it impossible to quantify the amount of the C_{44} component derived from the fat. Three extracts (NRA8 606 2164, NRA8 606 2166 and NRA4) contained distributions which were very characteristic of porcine fats, with relatively low abundances of the C_{48} and C_{54} components. The extract from NRA 8 606 2163 appeared to represent a mixture of fats, which may include porcine adipose due to the relatively low abundance of the C_{54} component. However, the C_{46} to C_{50} components were somewhat more abundant that would be expected in porcine fats. None of the extracts from Eton Lake End Road were identified as having an ovine origin.

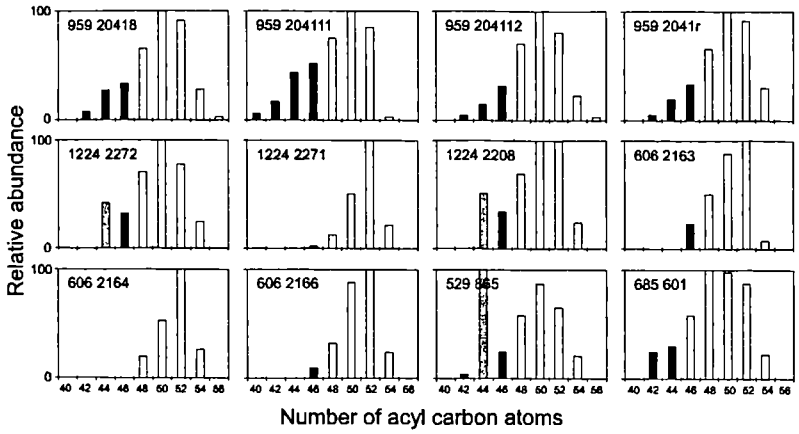


Figure 5.26 Histograms showing the carbon number distributions of triacylglycerols (acyl carbon number) in total lipid extracts of Late Neolithic/Early Bronze Age potsherds recovered from excavations at Lake End Road, Eton.

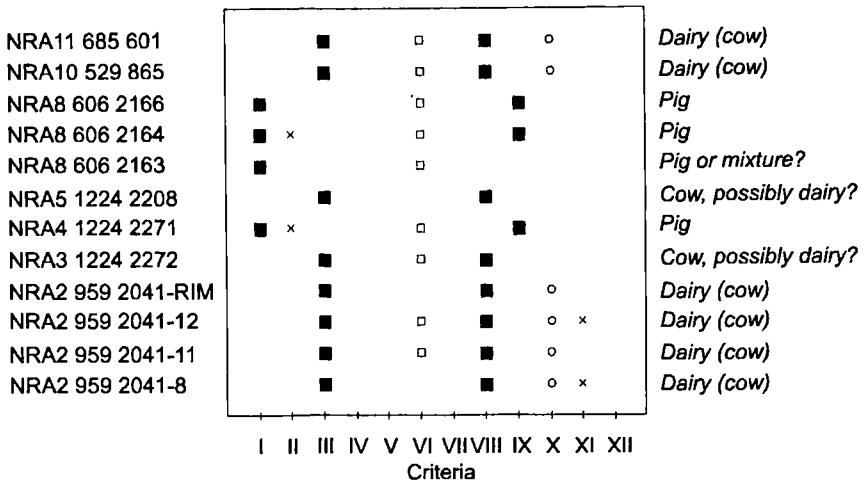


Figure 5.27 Characteristics of intact triacylglycerol distributions in archaeological fats from Eton Lake End Road and suggested origins for the remnant fats based upon comparisons with triacylglycerol distributions in reference fats.

5.3.3.4 Eton Rowing Lake (early Neolithic)

The 16 extracts studied from Eton Rowing Lake all comprised broad distributions of intact triacylglycerols, with none resembling the distinctive distribution of non-ruminant fats (Fig. 5.28; Table 10, Appendix 5, p. 384). Four extracts, from samples 1, 8, 12 and 13a, resembled ovine adipose (Fig. 5.29).

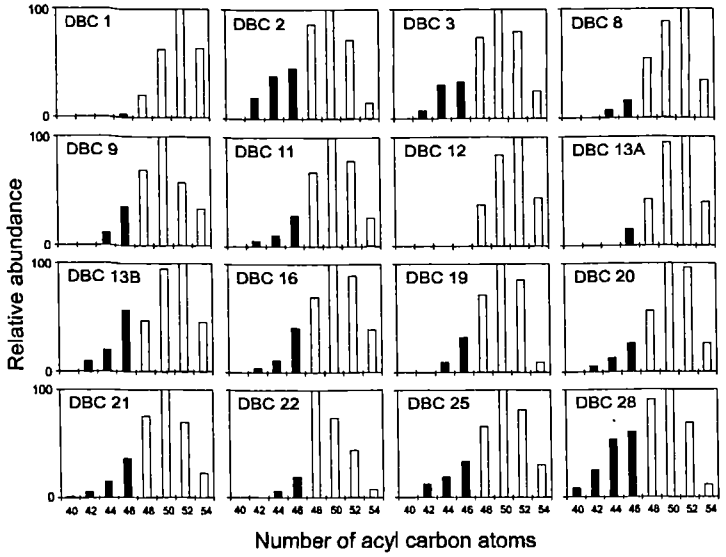


Figure 5.28 Histograms showing the carbon number distributions of triacylglycerols (acyl carbon number) in total lipid extracts of potsherds from the Early Neolithic vessels recovered from excavations at Eton Rowing Lake.

The extract from sample 22 comprised a relatively high abundance of the C_{48} component and a low abundance of C_{54}, characteristic of equine adipose fat. Nine extracts were identified as having a dairy origin, all considered to be from a bovine source, and the extracts from samples 9 and 19 comprised triacylglycerol distributions not dissimilar to bovine adipose. Thus, all of the extracts appear to derive from a ruminant source, with the possible exception of sample 22 which resembles horse adipose. The dairy fats appear to derive from a bovine source, and thus although ovine species have been identified at the site they do not appear to have been exploited for their milk.

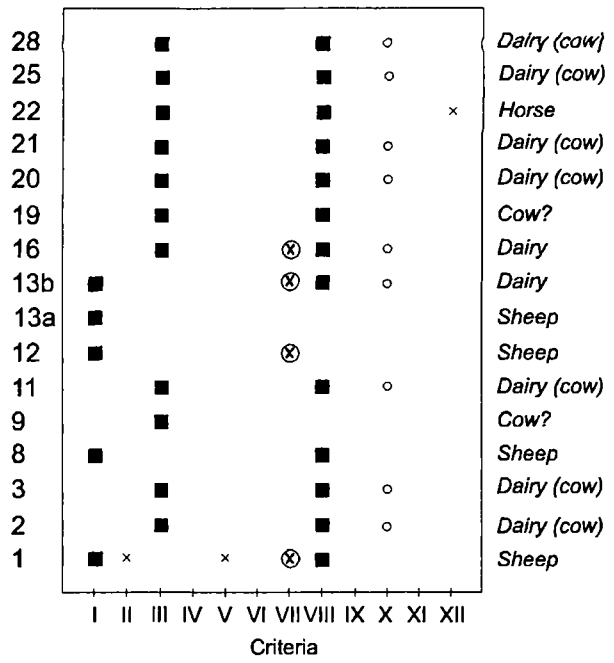


Figure 5.29 Characteristics of intact triacylglycerol distributions in archaeological fats from Eton Rowing Lake and suggested origins for the remnant fats based upon comparisons with triacylglycerol distributions in reference fats.

5.3.3.5 Upper Ninepence (early-late Neolithic)

Considering the age of the Walton site, the preservation afforded to these extracts, both from absorbed and carbonised surface residues, is remarkable (Fig. 5.30; Table 11, Appendix 5, p. 385). The triacylglycerol distributions are extremely uniform between the different vessel types, with the two Peterborough ware extracts comprising a relatively broad range of components similar to samples 33, 38 and 39 from the Grooved ware. In contrast, the extracts from Grooved ware vessels 66 and 68 more closely resemble non-ruminant, e.g. porcine fats [shown in Fig. 5.3(a)], due to the relatively low abundances of

the C_{44} to C_{48} and the C_{54} components. The remainder have been characterised as dairy fats from a bovine source (Fig. 5.31). It is notable that the extracts characterised as dairy fats from the Peterborough and Grooved ware sherds are similar, but not exactly the same, on account of the fact that the lower carbon-number triacylglycerols (C_{42} to C_{48}) in the Peterborough ware are in lower abundance than the corresponding components in the Grooved ware. Since the Peterborough ware sherds are dated approximately 500 years earlier than the Grooved ware, it is probable that a higher degree of preferential decay has resulted in the loss of a greater proportion of the lower carbon-number components from the Peterborough ware.

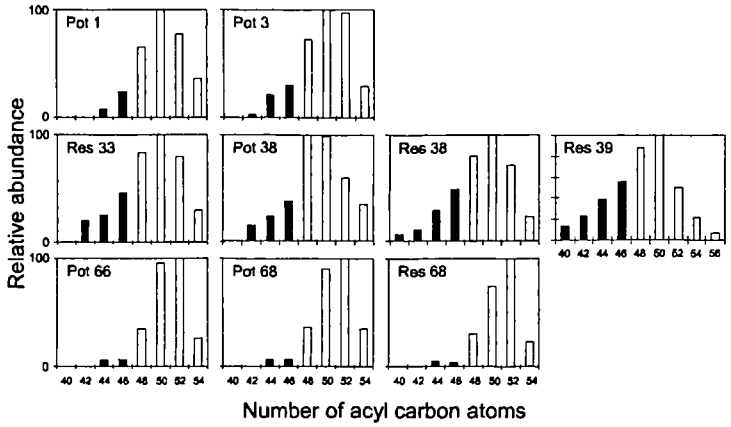


Figure 5.30 Histograms showing the carbon number distributions of triacylglycerols (acyl carbon number) in total lipid extracts from absorbed and carbonised residues from Walton sherds.

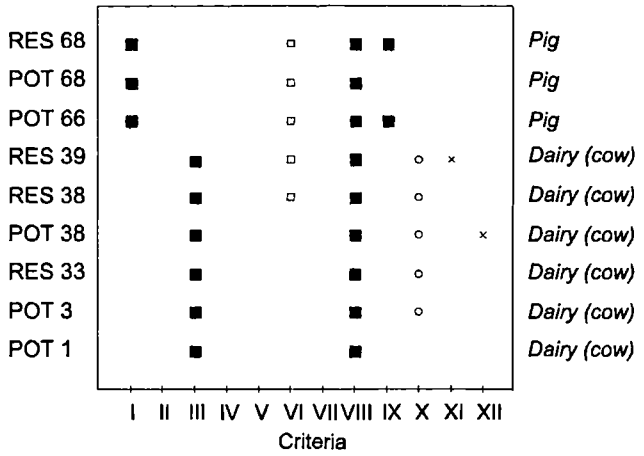


Figure 5.31 Characteristics of intact triacylglycerol distributions in archaeological fats from Walton and suggested origins for the remnant fats based upon comparisons with triacylglycerol distributions in reference fats.

5.3.4 Ethnographic vessels

The characteristics of the intact triacylglycerol distribution in the extract from ethnographic vessel A have been shown in Figure 5.13 (Table 12, Appendix 5, p. 385) compared with the saturated and combined saturated/mono-unsaturated fractions of the modern pig fat. The distribution in vessel A (Fig. 5.32) compares very well with the reference pig fat [Fig. 5.3 (b)]. The distribution is very characteristic on account of the high abundance of the C_{52} triacylglycerol compared with the other components present. No intact triacylglycerol components were present in the solvent extracts of sherds from ethnographic vessels B, C, F and G.

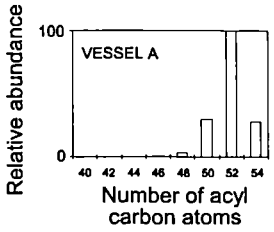


Figure 5.32 Histogram showing the carbon number distribution of triacylglycerols (acyl carbon number) in the total lipid extract of ethnographic vessel A.

5.3.5 Siberian horse fats

In the Siberian fats (Fig. 5.33) the relative abundances of the C_{46} and C_{48} are higher than in sample N26 and the modern reference horse fat shown in Figure 5.2 (k; Table 13, Appendix 5, p. 385). The data indicate that a higher proportion of the unsaturated components have been lost from the triacylglycerols in the Siberian fats, probably having been hydrolysed to the corresponding hydroxy acid which is present in very high abundance in these remnant fats.

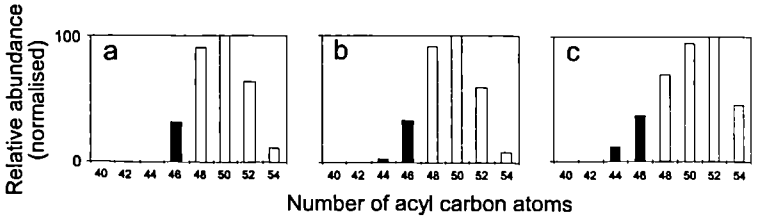


Figure 5.33 Histogram showing the distributions of triacylglycerols (of equal acyl carbon number) in the total lipid extracts of (a) internal (peritoneal), (b) subcutaneous fats, and (c) sacrum muscle, from the Siberian horses.

Samples from the stomach lining and the skin of the Siberian horses display similar distributions of intact triacylglycerols despite the fact that they derive from internal and external compartments of the animals body. The muscle attached to the sacrum was also analysed and displayed a somewhat different distribution of intact triacylglycerols, ranging between C_{44} and C_{54} and comprising high abundances of both the C_{50} and C_{52} and a relatively high C_{54}. This distribution is comparable with the bovine adipose fat seen in Figure 5.2(c) but very different from the more highly saturated fractions of the subcutaneous equine reference fats. The sample analysed was brown and, although crumbly in texture did not resemble adipocere and thus the distribution probably reflects the extremely high degree of preservation afforded to the horse meat.

5.4 Discussion

It has previously been observed that in remnant archaeological fats the intact acyl lipids comprising polyunsaturated components are not preserved, and those containing monounsaturated components are frequently present in much diminished abundance. Considering their abundance in fresh fats these components are clearly degraded in preference to their saturated counterparts. Therefore, in this chapter we have estimated the distributions of triacylglycerols which would remain following diagenetic alteration of natural fats and olive oil based upon the distributions of the more resistant saturated triacylglycerols. Several distinguishing factors have been identified in the saturated and monounsaturated fractions of animals believed to have been the major domesticated species in antiquity, including:

(i) The distributions of triacylglycerols in the remnant ruminant adipose and dairy fats are distinguished by the presence of shorter-chain components (C_{40} to C_{44});

(ii) Sheep and deer adipose are very similar in composition and it is not possible to reliably distinguish between them based upon the distributions of their intact triacylglycerols;

(iii) Ruminant and non-ruminant adipose fats have a similar range of components, however, non-ruminant distributions are dominated by the presence of only one or two compounds; the saturated C_{48} components predominate in the poultry fats, while the C_{50} and C_{52} components predominate in the porcine fats. Ruminant fats comprise a broader unimodal distribution, with a generally higher abundance of the C_{46}, C_{48} and C_{54} triacylglycerols;

(iv) The saturated fraction of horse fat is quite distinctive, but when a proportion of unsaturated triacylglycerols remain, the distribution is not easily distinguishable from bovine adipose fat;

(v) No distinction could be made between chicken and goose fats due to the similarity of their triacylglycerol compositions;

(vi) No saturated triacylglycerols could be fractionated from the olive oil, reflecting the high degree of unsaturation of the oil, however, the triacylglycerol components comprising one unsaturated moiety were fractionated and analysed by GC to reveal a very distinctive distribution, unrecognisable from that of intact olive oil.

The distinction between ovine and bovine adipose would appear to be relatively straight forward, based upon the C_{50}:C_{52} ratio and also upon a significantly higher abundance of C_{54} in ovine adipose. However, it was observed that dependant upon the extent of decay, measured by the loss of unsaturated moieties, the distribution of triacylglycerols in bovine adipose may have a greater or lesser abundance of the C_{52} component. Since it is difficult to assess the extent to which an archaeological fat has decayed, a remnant fat with a higher abundance of the C_{52} component could be mistakenly assigned as deriving from an ovine origin [Fig. 5.2(d) and (e)] when in fact it represents a well-preserved bovine fat [Fig. 5.2(a), (b) and (c)]. In addition, the possibility of the preferential cleavage of shorter-chain acyl moieties from triacylglycerols may complicate the use of relative abundances in assigning origin; higher carbon-number components may become predominant over their lower molecular weight counterparts due to the factors discussed in Section 5.1.4. This

eventuality could be assessed by the accelerated laboratory decay of reference fats under controlled conditions.

In the ruminant fats, a higher proportion of the total fat eluted in the upper fractions of the TLC plate than for non-ruminant fats, corresponding to the higher degree of saturation in the ruminant fats. This may account for: (i) the frequency with which we observe ruminant fats absorbed in pottery vessels, and (ii) the quantity in which they are preserved ($\mu g\ g^{-1}$ of powdered sherd) due to the greater potential for decay of the more susceptible mono- and polyunsaturated triacylglycerols which are more abundant in non-ruminant fats.

Based upon these characteristics of the intact triacylglycerol distributions, the majority of remnant animal fats from the archaeological assemblages have been assigned as adipose or dairy fats derived from one or other of the major domesticated species. The West Cotton extracts derive largely from sheep adipose fats, although the pottery vessels appear to have been used to process a variety of commodities, including porcine fats or meat and dairy products. The residues identified as having a dairy origin based upon the presence of lower-carbon-number components can be divided into two groups, distinguished by the relative abundances of the C_{50} and C_{52} components. The laboratory decay of milk fat (described in Chapter 7) indicates that degraded dairy fats from ovine animals contain a higher relative abundance of the C_{52} component, whilst on-going decay experiments would suggest that the C_{50} is more abundant in decayed bovine milk and butter fats. These observations mirror the differences seen between ovine and bovine adipose fats. Based on this criterion, dairy fats identified in the West Cotton vessels appear to have derived from both bovine and ovine sources. No bovine adipose fats have been unambiguously identified in the West Cotton vessels.

The data from the Stanwick pottery also suggest the exploitation of animal fats from various origins. Ovine adipose fats occur most frequently and 3 extracts have been tentatively identified as mixtures of porcine and ovine fats, although no residues exhibit the distinct characteristics of pure porcine fats. Dairy fats are common and derive from both ovine and bovine sources although bovine dairy fats appear to predominate.

Conversely, from the late Saxon site of Wicken Bonhunt, the residues indicate that only a very limited range of animal products were processed, since only ovine and porcine adipose fats have been identified. The ovine fats are probably all derived from sheep, as indicated from horn core evidence from the site.

Due to the poor preservation of intact acyl lipids in the Botai residues, triacylglycerols were only detected in two of the samples, however, one of these yielded a distribution comparable with degraded horse adipose, as would have been anticipated from a site at which horses formed an important part of the culture. The identity of the other residues may be realised once all of the chemical criteria are considered in combination.

The pottery from both Yarnton sites yielded numerous residues with triacylglycerol distributions characteristic of dairy fats, both from ovine and bovine sources. Adipose fats from porcine, ovine and bovine animals were also identified at the flood plain site. Pottery from the Cresswell field site yielded residues characteristic of ovine adipose fat, however, no porcine fats were identified. Dairy fats appeared to be mainly from a bovine source, indicating the exploitation of ovine animals for their meat and milk and bovines predominantly for milk production.

At Eton Rowing Lake, distinctions have been drawn between ovine adipose and bovine dairy fats, although no ovine dairy fats or porcine fats have been recognised based upon triacylglycerol distributions. The dependence appears to have been primarily on ovine meat, although one residue has been tentatively identified as horse fat/meat. The Lake End Road vessels also yielded numerous residues characteristic of bovine dairy fats, however, in contrast to the Rowing Lake site the predominant source of fat and meat appears to be from porcine animals.

The Peterborough and Grooved ware vessels from Walton yielded abundant intact acyl lipids, with the majority of the remnant fats identified as having a dairy origin. Grooved ware residues have been assigned as porcine adipose fats and bovine dairy fats, whilst all the Peterborough ware contained the same fat type, identified as bovine dairy fat. None of

the residues from these vessels exhibited triacylglycerol distributions characteristic of ovine fats.

Comparison of the distributions of di- and triacylglycerols in the archaeological fats has shown that the occurrence of different diacylglycerol components correlate with different distributions of triacylglycerols, and thus may be used to support differences between different fats. The distributions of diacylglycerols in archaeological fats have been found to fall within one of three groups, C_{32} to C_{36}, C_{30} to C_{36} and C_{28} to $C_{30/36}$, inclusive. These distributions correspond to distributions in porcine, ovine/bovine adipose and dairy fats, respectively. The distributions of diacylglycerols in archaeological fats are shown in Chapter 8.

The differences between intact triacylglycerol distributions in fresh and diagenetically altered natural fats considered in this Chapter has enabled remnant fats to be assigned to a particular animal fat origin. The reliability of this criterion will be tested when combined with the data discussed in the preceding three chapters. The use of this criterion is inevitably dependant upon the degree of preservation afforded to the organic residues being studied, since triacylglycerols are generally preserved in low abundance, e.g. compared to free fatty acids. At some sites where conditions are particularly extreme, for example Qasr Ibrîm in Egypt, triacylglycerols have not been detected intact, due to a combination of factors probably linked to the desiccating nature of the burial environment (H. Bland, pers. comm.). However, in general, the frequency of recovery of intact acyl lipid from vessels excavated from UK sites is high.

Preferential hydrolysis (due to the reasons discussed in Section 5.1.4) and dissolution of the resultant free fatty acids may significantly alter the original triacylglycerol profile of natural fats and oils (Section 7.5.2). However, this work has shown that differences between the triacylglycerol distributions in remnant fats can be used to distinguish between the main groups of domesticated animals based upon comparisons with the distributions of saturated triacylglycerols in modern fats. Thus, where intact triacylglycerol components are preserved in ancient fats, their distribution can provide information about the source of the fat, even though they no longer resemble their original profile.

CHAPTER 6
Stable Carbon Isotope Ratios

6.1 Introduction

6.1.1 Previous studies and considerations for utilising the natural variation in $\delta^{13}C$

'Isotope archaeology', i.e. the use of stable isotopes in archaeological investigations, is a relatively recent application which has focused largely on the use of bulk $\delta^{13}C$ and $\delta^{15}N$ measurements, providing new information intractable using traditional archaeological techniques. The first applications in archaeology utilised the difference in $\delta^{13}C$ values between C_3 and C_4 plant materials in order to assess the relative contributions of these plant types in the diets of ancient peoples (Vogel and van der Merwe, 1977; Jones *et al.*, 1979; Teeri and Schoeller, 1979; Tieszen *et al.*, 1979). Analyses have also enabled isotopic signals from marine and terrestrial sources to be distinguished as components of diet since the heavier isotope (^{13}C) is approximately 5-7‰ more abundant in the tissues of animals that consume marine foods (Chisholm *et al.*, 1982; Ostrom and Fry, 1993) and applications have included the investigation of human migrations, status and social structure (Sealy and van der Merwe, 1986; Murray and Schoeninger, 1988).

Applications of stable carbon isotope analyses in animal nutrition and metabolism studies are numerous (e.g. Boutton *et al.*, 1988) since the isotopic compositions of food and fluids ingested by animals have a strong influence on the isotopic compositions of the tissues they synthesise. However, the precise relationship between the isotopic compositions of ingested materials and any particular tissue or molecular component is complex, responding to changes in nutritional status, biosynthetic pathway (as discussed in Chapter 1) and turnover rate of the tissue. Field studies have been conducted in order to elucidate the complexities of how different biochemical fractions translate into consumer tissues in food chains. Attempts have been made to show the offset in $\delta^{13}C$ values between different trophic levels, however these depend upon the particular biochemical fraction, species type, diet and other environmental factors and therefore can only be broadly estimated. Lee-Thorpe (1989) calculated differences in bulk $\delta^{13}C$ values between the vegetation, herbivore and carnivore trophic levels based upon measurements of meat, collagen and apatite, noting an enrichment in ^{13}C in species further up the food chain. Feeding studies have been carried out to elucidate the relationships between different levels of the food chain and to establish to what extent different fractions in the diet are routed or scrambled

to particular tissues in consumers (e.g. Ambrose and Norr, 1993), with initial findings suggesting some degree of routing of dietary components to specific body tissues.

In our study of animals raised in antiquity we are anticipating that the diets of farmed animals would have been relatively constant, mainly comprising C_3 grasses and forage materials. Based on this assumption, the dietary contribution of $\delta^{13}C$ values to tissues such as adipose fat would be relatively constant (DeNiro and Epstein, 1978). However, variation may arise particularly in non-ruminant domesticates due to food supplements (e.g. from domestic waste such as whey left over from cheese production or meat scraps) which would contribute a larger protein component to the diet. In non-ruminant animals, the direct routing of dietary fats to storage organs such as adipose fats means that the isotopic signal of the dietary lipids is retained and will be reflected in the isotopic composition of the tissue (Section 1.9.2), although the situation is complex and varies between species. A recent study of the stable carbon isotope ratios of fatty acids in the body fat of Redhead ducks strongly indicated that fatty acids in the diet are not the sole contributor to adipose tissues and that the ducks also synthesise fatty acids from other fractions in their diet such as carbohydrates and proteins which result in more positive $\delta^{13}C$ values for the tissue fatty acids (Hammer *et al.*, 1998).

6.1.2 Stable isotope analyses of archaeological pottery residues

Morton and Schwarcz (1985) first applied stable isotope ($\delta^{13}C$ and $\delta^{15}N$) determinations to the study of residues associated with archaeological ceramics, using bulk measurements to examine the $\delta^{13}C$ values of carbonised material thought to originate from maize. The first application of compound-specific stable carbon isotope measurements to archaeological samples was reported by Evershed *et al.* (1994). The $\delta^{13}C$ values obtained for individual higher plant leaf wax components in solvent extracts of pottery vessels from the Raunds area project, Northamptonshire, confirmed that the lipids being investigated were of C_3 origin. The distributions of components were consistent with the lipids in the potsherds having derived from *Brassica* species, such as cabbage. The latter study utilised GC-C-IRMS, which allows the isotope ratios of individual compounds within a mixture to be determined (Santrock *et al.*, 1985). The development of this technique increased the specificity of stable isotope ratio studies, enabling for example, the use of isotopic tracers

in the Life and Environmental Sciences. Compound specific analyses have proven particularly advantageous over bulk analyses in the study of diagenetically altered samples due to the fact that bulk $\delta^{13}C$ values will alter over time as a result of the preferential loss of labile components, e.g. polysaccharides, resulting in the depletion of heavier carbon.

Workers in our laboratory first observed the isotopic distinction between remnant fats of ruminant and non-ruminant origin based on the stable carbon isotope composition of fatty acids in sherds from a small assemblage of lamps and 'dripping' dishes from a medieval site at Causeway Lane, Leicestershire (Mottram, 1995; Mottram *et al.*, 1999). Analyses of fresh lamb and pork fat indicated that fatty acids in ruminant fats are isotopically lighter, by approximately 4‰ and 7‰ for the $C_{16:0}$ and $C_{18:0}$ components, respectively, than fatty acids in non-ruminant fats. The variation is believed to result from fundamental differences in metabolic factors and dietary preferences between the species (Koch *et al.*, 1994). The data obtained clearly enabled the distinction of fats from two different animal origins in the two vessel types, indicating the use of the lamps in burning ruminant tallow and the 'dripping' dishes for the collection of non-ruminant, e.g. porcine fats, perhaps during spit roasting. The isotopic analysis of the extract from a 'cauldron' from the same assemblage gave $\delta^{13}C$ values which were intermediate between those obtained for the lamps and the 'dripping' dishes, indicating that the vessel had once been used to process a mixture of ruminant and non-ruminant animal products, which is quite consistent with a large vessel of this type (Mottram, 1995; Mottram *et al.*, 1999).

Preliminary work was carried out to investigate the potential of stable carbon isotopes in the investigation of the origins of remnant animal fat residues in Late Saxon/early medieval vessels from West Cotton, Northamptonshire (Charters, 1996). Extracts of four vessels were studied, including a shelly ware jar (RP78 rim) and three spouted bowls (RP72, 93 spout and 94 rim/body). The mean $\delta^{13}C$ values obtained for the fatty acids in the bowls differed by approximately 4‰ from the values obtained for fatty acids in the jar. The extracts from RP93, 94 and 72 were interpreted as deriving from a ruminant origin, however the extract from RP78 was thought to represent a mixture of fats from different origins since the $\delta^{13}C$ values were intermediate between those measured for reference ruminant and non-ruminant adipose fats. The studies by Mottram (1995) and Charters

(1996), albeit on a relatively small number of samples, were the first to recognise that differences between the stable carbon isotope compositions of remnant fats could be used to make distinctions between archaeological fats of different animal origins, and have provided the basis for the work described in this Chapter.

6.1.3 Measurement of $\delta^{13}C$ values

The lower trace in Figure 6.1 shows the baseline resolution obtained routinely by GC analysis of FAMEs on a 50 m CP WAX 52 CB fused silica capillary column. The upper trace is a ratio of the *m/z* 45/44 ions in the sample detected by the GC-combustion-isotope ratio mass spectrometer (GC-C-IRMS). Operating conditions are given in Section 9.2.3. The stable carbon isotope ratios are measured as the relative difference between the isotopic ratios of the sample and standard gases, thus adopting the delta (δ) notation (McKinney *et al.*, 1950):

$$\delta^{13}C\ (‰) = \frac{[R_{sample} - R_{standard}]}{R_{standard}} \times 10^3$$

where the $\delta^{13}C$ is the parts per thousand difference between the ^{13}C content of the sample and that of the standard and R is the *m/z* 45/44 ratio of the sample or standard gas.

$\delta^{13}C$ values are expressed relative to VPDB. This standard has been assigned a $\delta^{13}C$ value of 0‰, thus the notation of the $\delta^{13}C$ value indicates whether the sample has a higher or lower $^{13}C/^{12}C$ ratio than VPDB. Samples are run in triplicate and the mean values obtained corrected for the additional carbon of the derivatising agent, BF_3MeOH. The $\delta^{13}C$ value of the BF_3MeOH has previously been measured at -41.4‰ ± 0.5. Modern fats and oils used as reference samples are corrected for the change in atmospheric CO_2 which has occurred since the Industrial Revolution (according to Friedli *et al.*, 1986).

Bulk isotope values have been measured on homogenised plant materials and whey using an NC 2500 elemental analyser coupled with the Finnigan MAT Delta-S isotope ratio mass spectrometer *via* an open split interface.

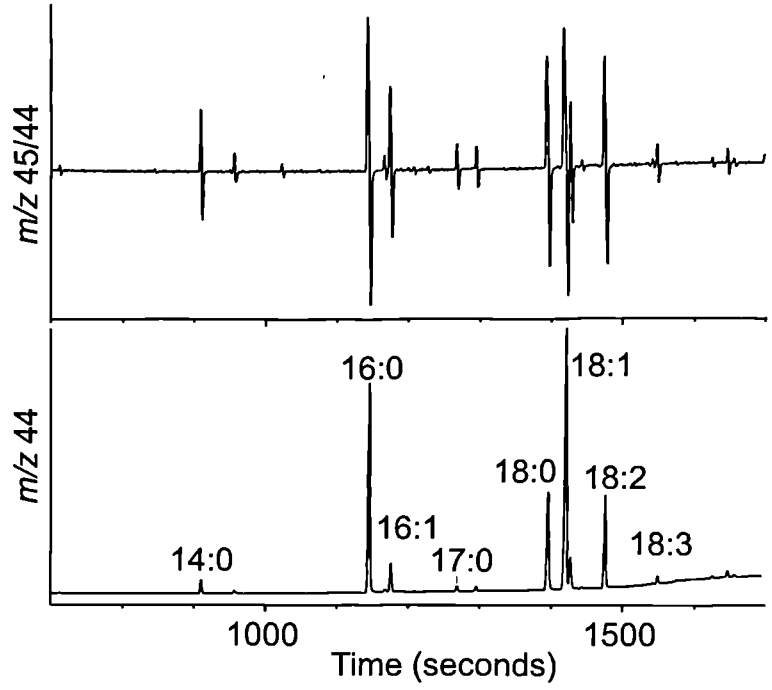


Figure 6.1 Partial *m/z* 44 and *m/z* 45/44 traces obtained by GC-C-IRMS analysis of fatty acids (as their methyl ester derivatives) in pig adipose fat (sample P3T121). GC-C-IRMS operating conditions and chromatographic conditions are as described in Section 9.2.3.

Presented within this chapter are the ranges of $\delta^{13}C$ values in the body and milk fats of animals known to have been the major domesticated species in antiquity, noting differences between individuals of the same species and between different species. Since the diets of the reference animals are known it is possible to test the relationship between the stable carbon isotope ratios of lipid components in the diet and in the animal fats. The study also constitutes by far the largest study of $\delta^{13}C$ values of individual lipids from archaeological pottery to date, and will thus enable an assessment of the usefulness of the chemical information which can be obtained from this type of analysis. The study of prehistoric pot extracts will enable us to establish whether the distinction in the isotopic signals previously observed between fats of different origin in the medieval pottery from Causeway Lane (Mottram, 1995) is retained even in lipids from early Neolithic vessels, or whether use of this criteria is thwarted due to diagenetic alteration, contamination, differences in forage materials and breeds of animal, etc. over longer archaeological time scales.

6.2 Results of stable carbon isotope analysis of reference fats

The $\delta^{13}C$ values of the $C_{16:0}$ and $C_{18:0}$ fatty acids for modern reference fats from different species are shown in Figure 6.2. The numbers (n) of different reference fats analysed (in triplicate) were: pig adipose fat, n = 9; cow adipose fat, n = 4; sheep adipose fat, n = 7; chicken adipose fat, n = 8; cows' milk fat, n = 8; sheep milk fats, n = 2; horse adipose fat, n = 8; deer fat, n = 7. All the animals were raised on C_3 diets, isotopically representative of the archaeological period, and for this reason numbers of samples suitable for this work were limited.

From the data shown in Figure 6.2 the following points have been observed:

i) Adipose fats from the major ruminant (e.g. ovine and bovine) and non-ruminant (e.g. porcine) domesticates are distinguishable from one another by greater depletion in ^{13}C in the $C_{16:0}$ and $C_{18:0}$ fatty acids in ruminant fats. The mean $\delta^{13}C$ values obtained for adipose fats from the reference pig and sheep differ by 3.2‰ in the $C_{16:0}$ fatty acid and 6.1‰ in the $C_{18:0}$ fatty acid;

ii) The $C_{16:0}$ and $C_{18:0}$ fatty acids in porcine adipose fats and salt-water fish tissues show the least depleted $\delta^{13}C$ values of the reference fats analysed;

iii) The mean $\delta^{13}C$ values obtained for the cattle adipose fats are more depleted than the sheep adipose, by 0.6‰ and 1.2‰ for the $C_{16:0}$ and $C_{18:0}$ fatty acids, respectively;

iv) A distinction can be made between the $\delta^{13}C$ values of fatty acids in ruminant adipose and dairy fats, primarily based on the greater depletion of the $C_{18:0}$ fatty acid in dairy fats (ca. 2-3‰);

v) The mean $\delta^{13}C$ values obtained for the sheep milk are very similar to the mean values for the cow's milk;

vi) Depot fats from chicken and goose show almost identical $\delta^{13}C$ values for the $C_{18:0}$ fatty acid and a difference of only 0.4‰ between the mean values obtained for the $C_{16:0}$ fatty acid;

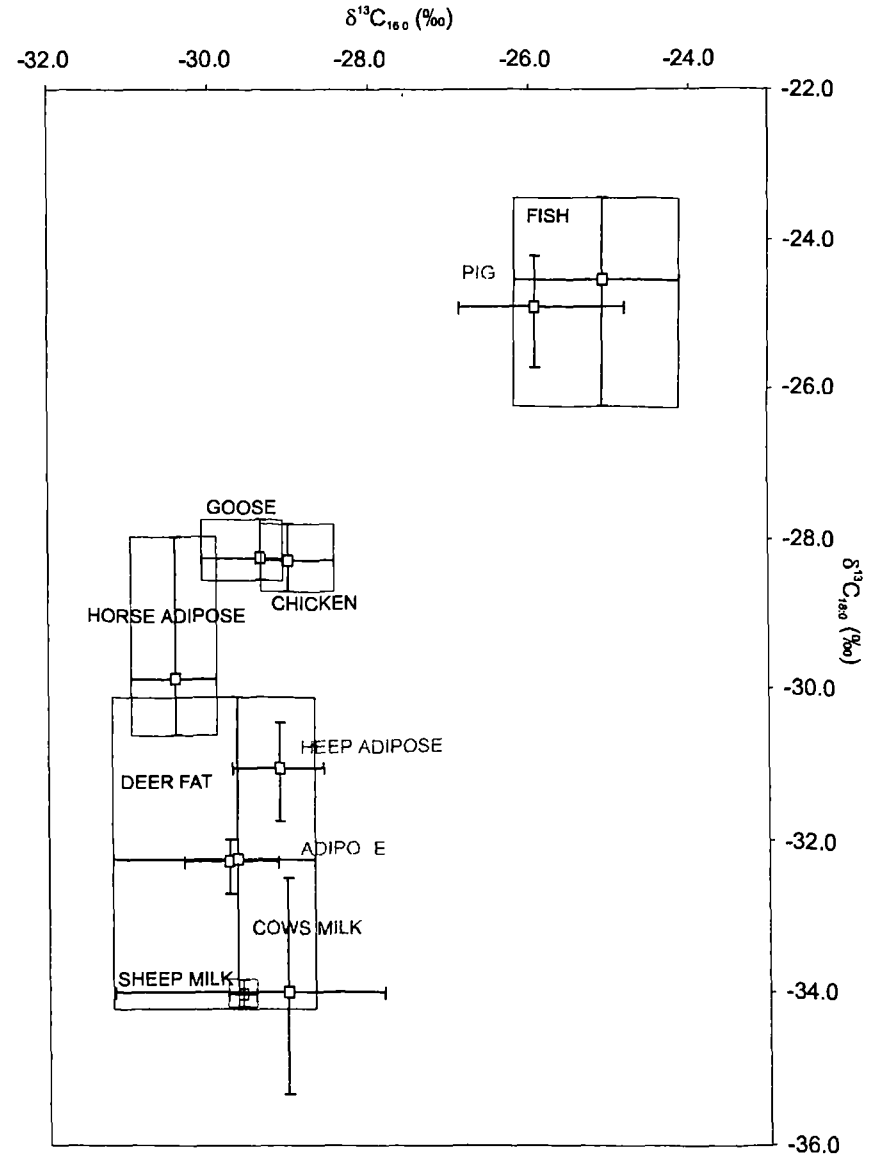


Figure 6.2 Plot of the $\delta^{13}C$ values for the major *n*-alkanoic acid ($C_{16:0}$ and $C_{18:0}$) components of the solvent extracts of modern reference fats. The $\delta^{13}C$ values of the individual fatty acids were determined exactly according to the conditions given in Woodbury *et al.* (1995) with corrections for the addition of the derivatising methyl carbon. The $\delta^{13}C$ values for the fatty acids in the reference fats have been corrected for the post-Industrial Revolution effects of fossil fuel burning which has decreased the $\delta^{13}C$ value of atmospheric CO_2 by approximately 1.2‰ over the past 130 years (Friedli *et al.*, 1986). The boxed fields encompass the ranges for reference animal fats with the ranges crossing at the arithmetic mean. Instrumental error is ± 0.3‰ and samples were run in triplicate. GC operating conditions are described in Section 9.2.3.

vii) The fatty acids in horse adipose are more depleted than the other non-ruminant fats, with mean $\delta^{13}C$ values of -30.4‰ and -29.8‰ for the $C_{16:0}$ and $C_{18:0}$ fatty acids, respectively;

viii) The mean $\delta^{13}C$ values of the deer fats are similar to the other ruminant adipose fats, however the range of values obtained is significantly greater. This variation is surprising due to the fact that all these animals were of the same breed and raised on the same pasture;

ix) The $\delta^{13}C$ values for the depot fats of individual animals are closely grouped (with the exception of deer fat), however in contrast, the range of $\delta^{13}C$ values obtained for the milk fats varied by up to 3.4‰ for the $C_{16:0}$ and 2.9‰ for the $C_{18:0}$ fatty acid.

6.3 Deducing the relationship between the stable carbon isotope composition of lipid components of adipose fats and diet

The mean $\delta^{13}C$ values for the major saturated and unsaturated fatty acids in the reference ruminant animal fats and their diets are plotted in Figures 6.3 and 6.4 and shown in Tables 1, 3 and 4, Appendix 6 (pp. 386-389). Data have been included from cows which were fed concentrates as a supplement to the diet in order to compare with the data from C_3 grass-fed animals. The fatty acids in adipose tissue from the concentrate-fed and the grass-fed cow differ by approximately 2‰, with the former reflecting the heavier isotopic composition of the concentrate supplement. For the silage and fresh grasses analysed, the bulk $\delta^{13}C$ value is significantly less depleted in ^{13}C, by approximately 6‰, than the individual $C_{16:0}$ and $C_{18:0}$ fatty acids. Since the majority of higher plant tissue is comprised of carbohydrate, with only ca. 7% lipid, the bulk value obtained reflects the isotopically heavier carbohydrate. $\delta^{13}C$ measurements for individual fatty acids in the grasses have shown that the $C_{14:0}$ is most depleted isotopically, followed by the $C_{16:0}$ and the $C_{18:0}$ fatty acids.

The variations in $\delta^{13}C$ values obtained for the individual fatty acids in the grasses and those from cow adipose tissue illustrate that the relationship between diet and tissue is extremely difficult to interpret. This is due to the complexity of the metabolic and physiological processes determining adipose fat formation in different animal species, as previously discussed in Chapter 1. The $C_{14:0}$, $C_{16:0}$ and $C_{18:0}$ in cow adipose tissue are generally less depleted than the same fatty acids in the diet indicating that a proportion of

these components are synthesised *de novo* and reflect a contribution from other sources of carbon in the diet, e.g. carbohydrate and protein. The relationship between diet and fat cannot be explored fully without examining the routing of different sources of dietary carbon and utilisation of stored carbon in the whole animal (*viz* DeNiro and Epstein, 1978). The $\delta^{13}C$ values for the sheep from Baker's farm fed some supplements to their diet are very similar to those for the grass-reared sheep from Brockley since the bulk of their diet was grass.

In ruminant animals there is a large (ca. 3‰) difference between the $\delta^{13}C$ values for the $C_{16:0}$ and $C_{18:0}$ fatty acids in adipose and an even larger difference of 4-6‰ between the same fatty acids in milk fat. This relatively large difference in the $\delta^{13}C$ values of the fatty acids indicates different sources for these components, i.e. the direct routing of dietary fatty acids and the synthesis of components in different organs of the body, e.g. liver, adipose, mammary gland, etc. from different precursors which may result in differing degrees of isotopic discrimination in fat synthesis. The direct routing of fatty acids in the formation of adipose fats is thought to be minor since the fat content of the diets of the major domesticated animals is relatively low (<5%) and thus the major portion of the fat deposited as adipose fat will be biosynthesised by the animal itself (Emery, 1980).

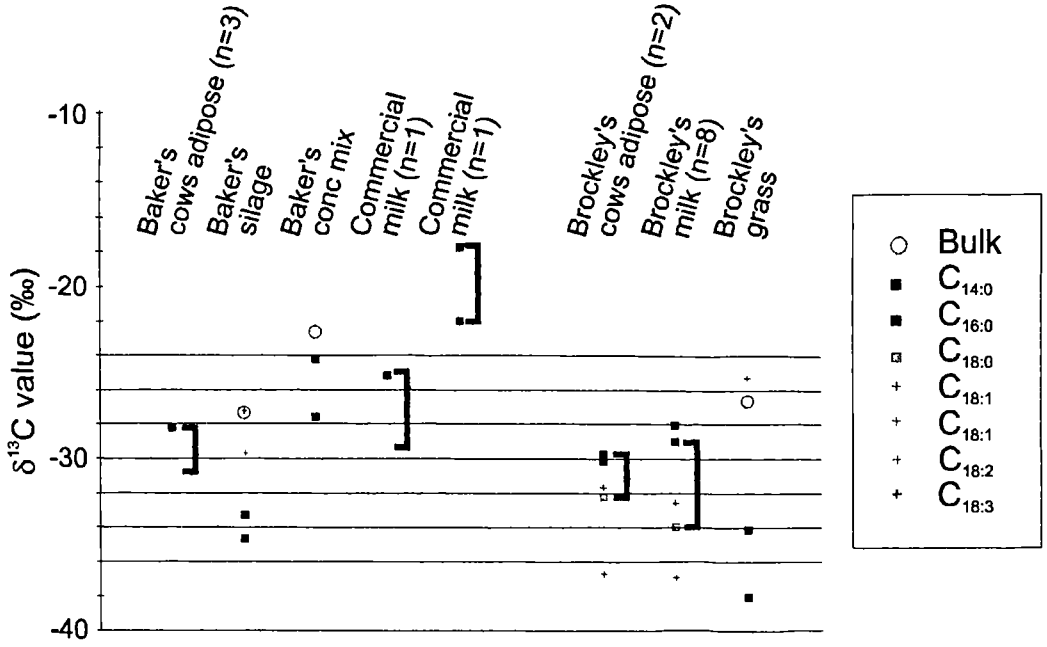


Figure 6.3 The relationship between carbon composition of the diet and bovine adipose and milk fatty acids (the variation between the major $C_{16:0}$ and $C_{18:0}$ fatty acids in adipose and milk fats is shown by the red bars).

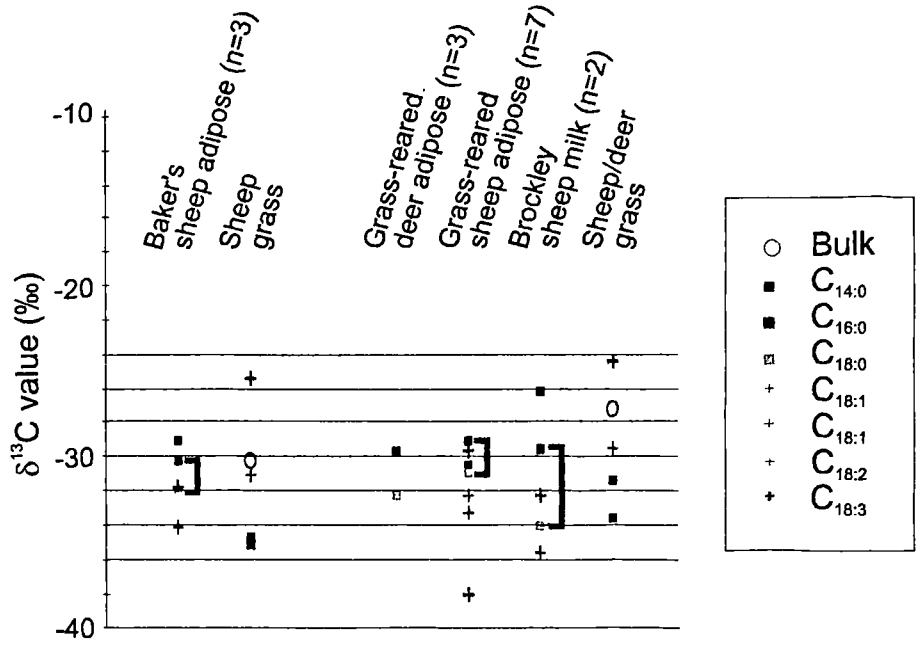


Figure 6.4 The relationship between carbon composition of the diet and ovine adipose and milk fatty acids (the variation between the major $C_{16:0}$ and $C_{18:0}$ fatty acids in adipose and milk fats is shown by the red bars).

In the cow adipose and milk samples, including those from concentrate-fed animals, the $C_{16:0}$ is less depleted in ^{13}C than the $C_{18:0}$ fatty acid. The $\delta^{13}C$ values of the $C_{18:0}$ fatty acids in cow's milk are depleted by approximately 2‰ relative to the $C_{18:0}$ fatty acid in adipose fat and may reflect a direct contribution from the more depleted $C_{14:0}$, $C_{16:0}$ (following chain elongation) and C_{18} fatty acids in the grass/forage (Fig. 6.3). The relative importance of these different contributions are discussed further in Section 6.3.1.2. The $\delta^{13}C$ values of fatty acids in milk from concentrate-fed animals are less depleted than milk from grass fed animals due to the influence of the supplements in their diet, however, the $C_{18:0}$ is still significantly more depleted in ^{13}C than the $C_{16:0}$. The $\delta^{13}C$ value of the $C_{18:0}$ fatty acid is also depleted relative to the $C_{16:0}$ by ca. 4‰ in sheep milk (Fig. 6.4). The difference between the $C_{16:0}$ and $C_{18:0}$ fatty acids in sheep adipose is of lower magnitude (ca. 2‰). The effect of diet on the composition of milk fats is clearly shown by the relatively large enrichment in ^{13}C (up to -18‰) of the fatty acids in dairy fats from cows fed concentrate supplements. Thus, there appears to be a distinction between the $\delta^{13}C$ values of milk and adipose from ruminant animals probably reflecting differences in the physiological and metabolic processes involved in their production.

Non-ruminant fats comprise fatty acids which are enriched in ^{13}C in the order $C_{14:0}<C_{16:0}<C_{18:0}$, with the $C_{18:0}$ the most enriched (Fig. 6.5; Table 2, Appendix 6, p. 388). However, in ruminant milk and adipose fats the opposite is the case, with the $C_{14:0}$ the most enriched fatty acid (Fig. 6.4). In non-ruminant body fats there is less variation (ca. 0.5-1‰) between the $\delta^{13}C$ values of the $C_{16:0}$ and $C_{18:0}$ fatty acids compared with ruminant fats. The data show that the fatty acids in the tissues are significantly more enriched than the fatty acids in the diet and more closely reflect the bulk $\delta^{13}C$ value obtained for the diet. The ~2-4‰ depletion in ^{13}C relative to the bulk diet is possibly due to discrimination against ^{13}C during *de novo* fat biosynthesis in non-ruminants.

The lipid components of the porcine fats are ca. 5‰ more enriched than that of the herbivores, probably reflecting several factors, including: i) the isotopic composition of the diet; ii) the proportion of protein components in the diet (e.g. meat protein); iii) the degree to which different animals rely on different fractions of the diet for energy metabolism [e.g. carnivores depend mainly on protein for energy metabolism, whereas herbivores and omnivores may use excess protein for energy (Krueger and Sullivan, 1984)]; iv) the presence of the rumen in herbivores which facilitates breakdown and absorption of complex organic materials, and v) the differences in metabolism by which different fractions in the diet are routed or scrambled into the production of body fats. Studies have suggested that carbon in dietary proteins is routed to collagen in rats (Chisholm *et al.*, 1982) indicating that some routing occurs rather than simple scrambling of all the different components in the diet (Schwarcz *et al.*, 1985; Spielmann *et al.*, 1990). However, at present, factors controlling the isotopic composition of tissues of different herbivorous and omnivorous animals are poorly understood.

Figure 6.5 shows that a distinct pattern exists in the relationship between the diet and adipose tissue of non-ruminant animals, with both the $C_{16:0}$ and $C_{18:0}$ fatty acids equally enriched in ^{13}C relative to the diet. The degree of enrichment is variable between species, with differences of ca. 1‰ between fatty acids in chicken feed and adipose fat, and up to 5‰ between fatty acids in pig feed and pig adipose fat.

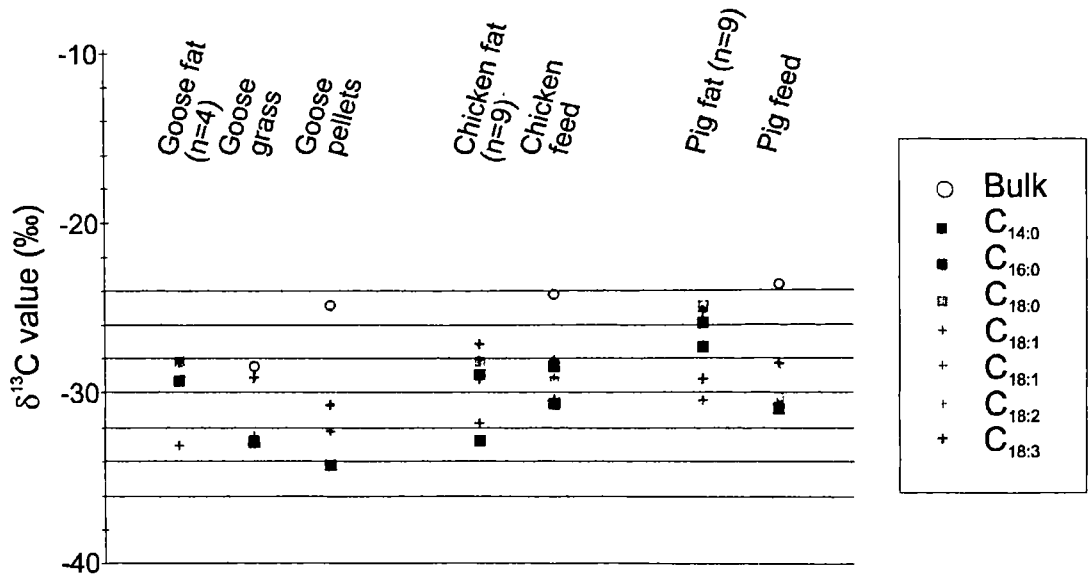


Figure 6.5 The relationship between carbon composition of the diet and fatty acids in non-ruminant adipose fats.

6.4 Stable carbon isotope composition of archaeological samples

Samples were selected for stable carbon isotope analysis on the basis that the overall distribution of lipid components resembled a degraded animal fat, and that they contained sufficient quantities of $C_{16:0}$ and $C_{18:0}$ fatty acids for analysis by GC-C-IRMS. Extracts in which leaf wax components were also identified were generally avoided in order to obtain pure animal fat signals. In order to assess the effect of mixtures of fats from different reference animals on the isotopic signal, theoretical mixing lines have been constructed according to Woodbury *et al.* (1995). The mixing curves were constructed taking into account both the relative proportions of the major *n*-alkanoic acids and the stable carbon isotope values of the acids present in the pure fats. The $\delta^{13}C$ values for mixtures of fats in varying proportions are plotted for comparison with the archaeological data.

6.4.1 Sites with well-documented faunal assemblages

6.4.1.1 West Cotton (Late Saxon/early medieval)

$\delta^{13}C$ values were obtained for the $C_{16:0}$ and $C_{18:0}$ fatty acids in the selected remnant fats from West Cotton. The data are plotted in Figure 6.6 (Table 5, Appendix 6, p. 390) together with $\delta^{13}C$ values obtained for the fats of modern equivalents of the domesticated animals represented in the faunal assemblage at West Cotton. Three archaeological fats, sample nos. RP4, 10 and 88, correspond closely with the data obtained for the reference

pig fats. The majority of the remainder contain fatty acids with $\delta^{13}C$ values which plot along the mixing curves between the reference ruminant and non-ruminant adipose fats and in the region of the reference ruminant fats. Several of the archaeological fats from West Cotton were found to correspond to the data obtained for the reference ruminant milk fats, including RP30, 60, 61, 86, 94 and WC30, distinguished by a lighter isotopic signal (mean≈-33‰) for the $C_{18:0}$ fatty acid. Two other archaeological fats, sample nos. RP72 and 91, cluster around the mixing curve between the reference milk and non-ruminant fats. Based on the distributions of lipid components, it had previously been assumed that the majority of remnant fats from West Cotton were derived from degraded adipose fats (Charters, 1996), probably of an ovine origin due to the high abundance of sheep bones recovered from the site. However, in the light of these new data, at least six remnant fats appear to derive from a dairy origin based on their stable carbon isotope compositions. WC30, the medieval 'top hat' vessel from West Cotton has been referred to previously (Section 1.4; Charters, 1996; Evershed *et al.*, 1992b) due to the abundance of short-chain fatty acids contained in the residue which are diagnostic of milk fats. It is very encouraging that the $\delta^{13}C$ values for the $C_{16:0}$ and $C_{18:0}$ fatty acids from this residue plot in the range for the reference milk fats since this validates the methods described herein. None of the other remnant fats from West Cotton contained such a high abundance of short-chain fatty acid components which could identify them as dairy fats, the only evidence indicating their dairy origin has been obtained through the stable carbon isotope analysis of their $C_{16:0}$ and $C_{18:0}$ fatty acid components.

The stable carbon isotope data obtained in the re-analysis of the selected West Cotton vessels previously studied by Charters (1996; Section 6.1.2) is comparable with the data initially obtained and the original interpretation still stands, with vessel RP78 derived from either a ruminant adipose origin or from a mixture of ruminant and non-ruminant fats. The results of the re-analysis also indicate that the fats from the spouted bowls all derive from ruminant animals, with $\delta^{13}C$ values correlating with the data obtained for the reference dairy fats. The data indicate a common function for these spouted bowls. Since subsamples of the same potsherds were re-extracted prior to stable carbon isotope analysis as part of this study, the close similarity of the results obtained in analyses by Charters (1996) and

the new data are very encouraging, validating the analytical procedures followed and illustrating the reproducibility of the compound-specific stable isotope analyses.

Figure 6.7 shows a plot of the stable isotope data compared with vessel form. Vessel function does not correlate well with form, although some observations can be made, e.g. both of the St Neot's Jars [refer to Charters (1996) for a detailed description] have shown isotope values which correspond with the reference ruminant adipose. Shelly ware jars were apparently used for a range of culinary functions since various sherds from these vessels have isotope values corresponding with ruminant adipose and dairy fats and non-ruminant fats; data from the Shelly ware bowl correspond with the non-ruminant reference fat.

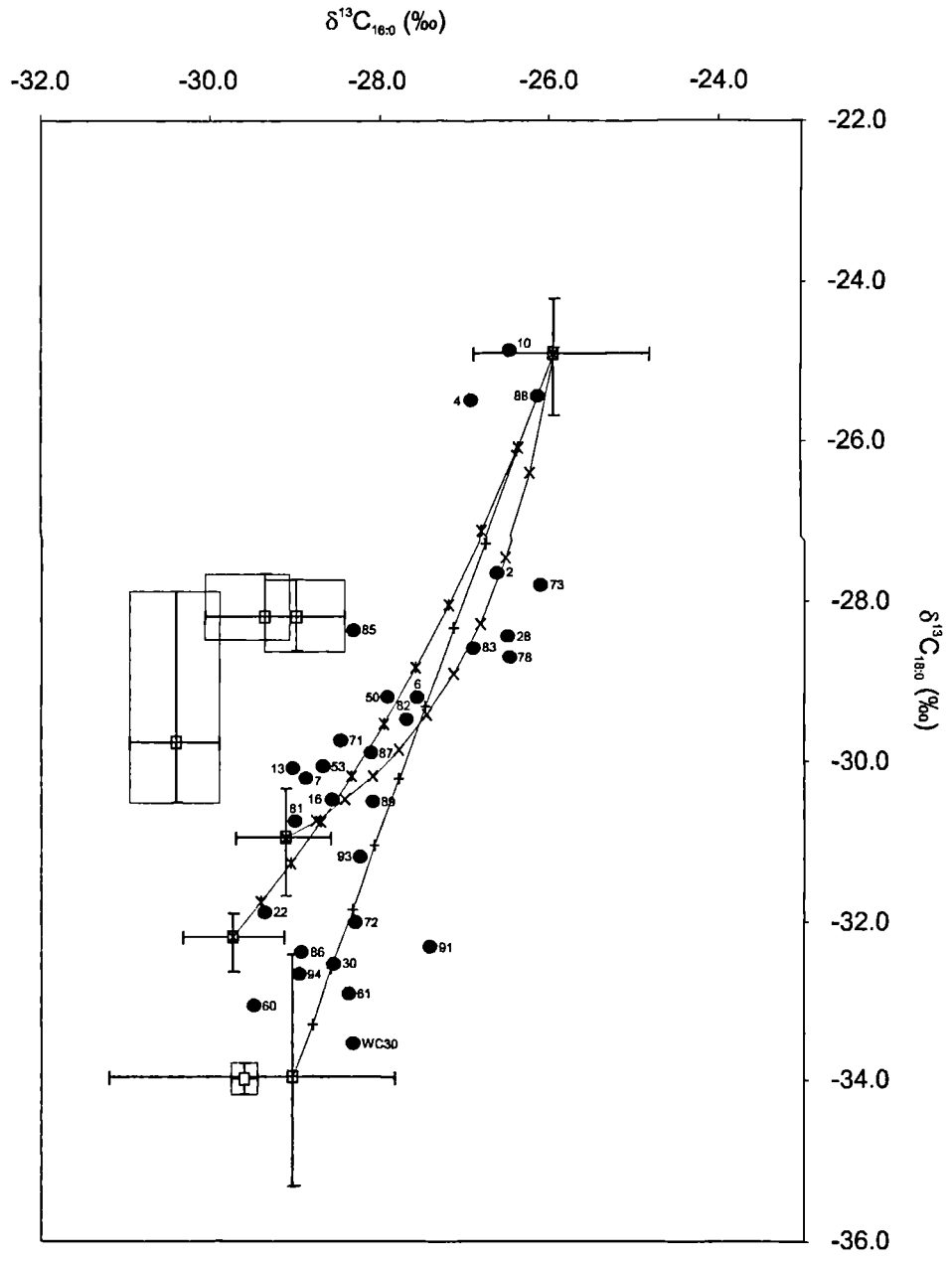


Figure 6.6 Plot of the $\delta^{13}C$ values of the major *n*-alkanoic acid components ($C_{16:0}$ and $C_{18:0}$) from the lipid extracts of potsherds from the Late Saxon/early medieval site of West Cotton, Northamptonshire. The blue-filled circles represent the archaeological fats; sample nos. are labelled. Mixing curves are as described in Appendix 7 (pp. 395-396). The mixing curves have been calculated to illustrate the $\delta^{13}C$ values which would result from the mixing of ovine and porcine fats (x), bovine and porcine fats (✱) and cow's milk/porcine fats (+) in the vessels.

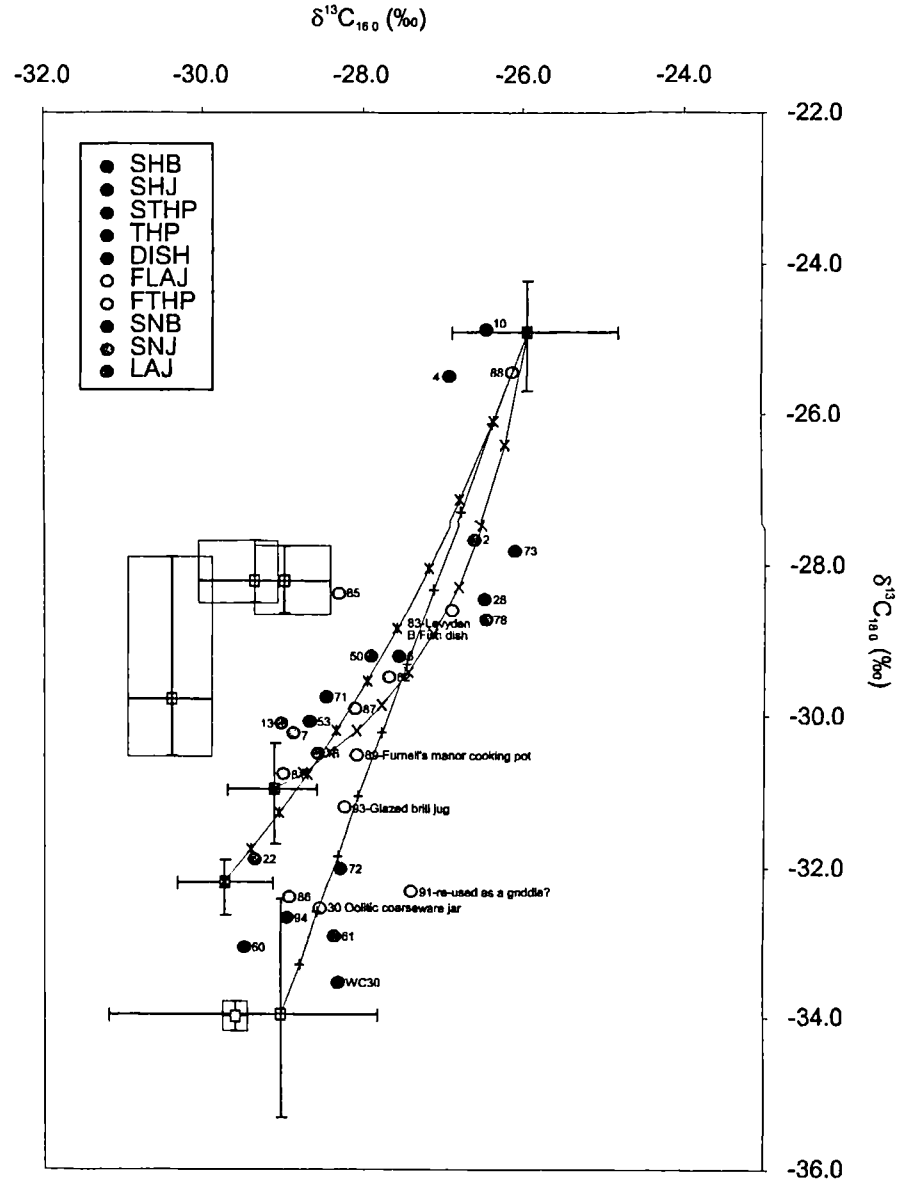


Figure 6.7 Plot of the $\delta^{13}C$ values of the $C_{16:0}$ and $C_{18:0}$ fatty acid components from the lipid extracts of potsherds from the Late Saxon/early medieval site of West Cotton, Northamptonshire, correlated with vessel form. The abbreviations denoting vessel form are as follows: SHB, shelly ware bowl; SHJ, shelly ware jar; STHP, shelly ware 'top hat' pot; THP, 'top hat ' pot; DISH, dish; FLAJ, Furnell's Manor Lyveden A ware jar; FTHP, Furnell's Manor 'top hat' pot; SNB, St. Neot's bowl; SNJ, St. Neot's jar, and LAJ, Lyveden A ware jar.

Some correlation can be observed between stable isotope data and date, including: i) the Late Saxon residues plot in the region of the ruminant adipose and dairy fats; ii) the early medieval pots, ca. 1100 to 1150 AD (site of earlier medieval settlement and manor) plot in the region of the ruminant adipose and in line with the mixing curve indicating mixtures of ruminant and non-ruminant fats, however there is no isotopic evidence for dairy or pure non-ruminant fats; iii) data from four sherds dating ca. 1150-1225 AD include two which plot closely together quite high up the mixing curve towards the reference porcine fats and two which plot together in the region of the dairy fats, and iv) all of the sherds dating between 1225 and 1300 AD (site of medieval manor and hamlet) plot in the region of the ruminant adipose fats. Unfortunately dates are not known for all of the sherds analysed, however, it appears that residues from both ruminant adipose and dairy fats are associated with all periods at West Cotton. A larger data set for the main periods would provide a clearer picture of changes or trends in vessel and commodity use.

6.4.1.2 Stanwick (Iron Age/Romano-British)

Figure 6.8 is a plot of the stable carbon isotope data obtained for the $C_{16:0}$ and $C_{18:0}$ fatty acids from archaeological fats from the Stanwick assemblage compared *with the same* reference fats and mixing curves as previously described. The data are shown in Table 6, Appendix 6 (p. 390). The clustering of archaeological fats clearly illustrates the predominance of ruminant adipose and dairy fats in these Romano-British and Iron Age sherds. In contrast to West Cotton, none of the remnant fats from Stanwick plot with the non-ruminant (e.g. porcine) reference fats, however, a number of the archaeological fats plot along the mixing curve between the ruminant and non-ruminant fats. Several of the fats appear to derive from a dairy origin due to their close correlation with data obtained for the modern reference milk fats, including ST193, 206 body, 197, 160, 194, 161 and 208. The reliability and wider application of the stable isotope approach is re-enforced by the data obtained from these analyses since the spread of $\delta^{13}C$ values from Stanwick, seen in Figure 6.8, mirrors that seen in Figure 6.6 for the West Cotton extracts, except for the notable absence of non-ruminant (e.g. porcine) fats amongst the Stanwick assemblage.

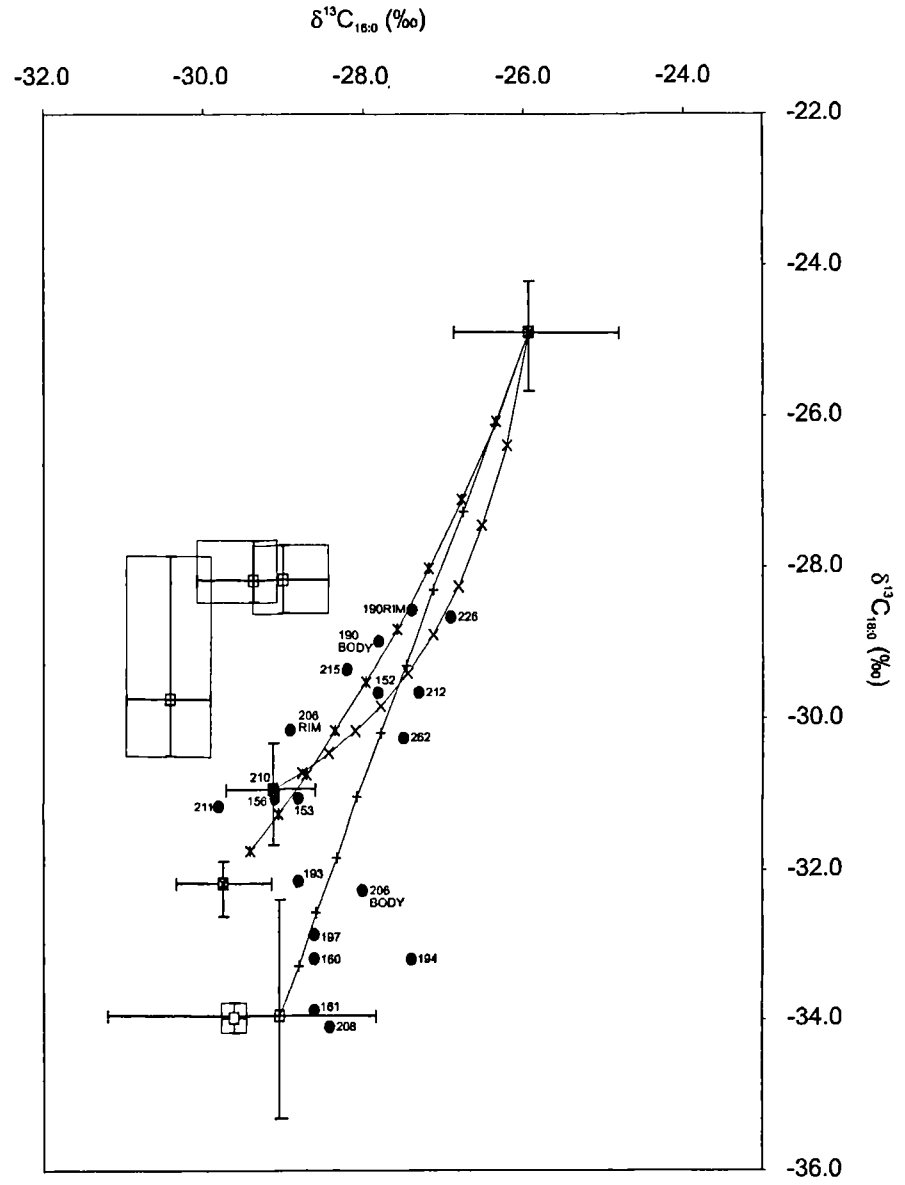


Figure 6.8 Plot of the $\delta^{13}C$ values of the major *n*-alkanoic acid components ($C_{16:0}$ and $C_{18:0}$) from the lipid extracts of potsherds from the Iron Age/Romano-British site of Stanwick, Northamptonshire, compared with data from modern reference fats.

The correlation of vessel form/fabric type and stable carbon isotope ratios of the fatty acids in the Stanwick vessels shown in Figure 6.9 illustrates that both Grogged ware bowls contain residues which plot with the reference dairy fats, and both sherds from the Grogged Channel-rim jar plot closely together on the mixing curve between the reference ruminant and non-ruminant adipose fats. The two Iron Age Channel-rim jars correlate with the reference dairy fats and both of the Iron Age jars plot in the region of the reference ovine adipose fats.

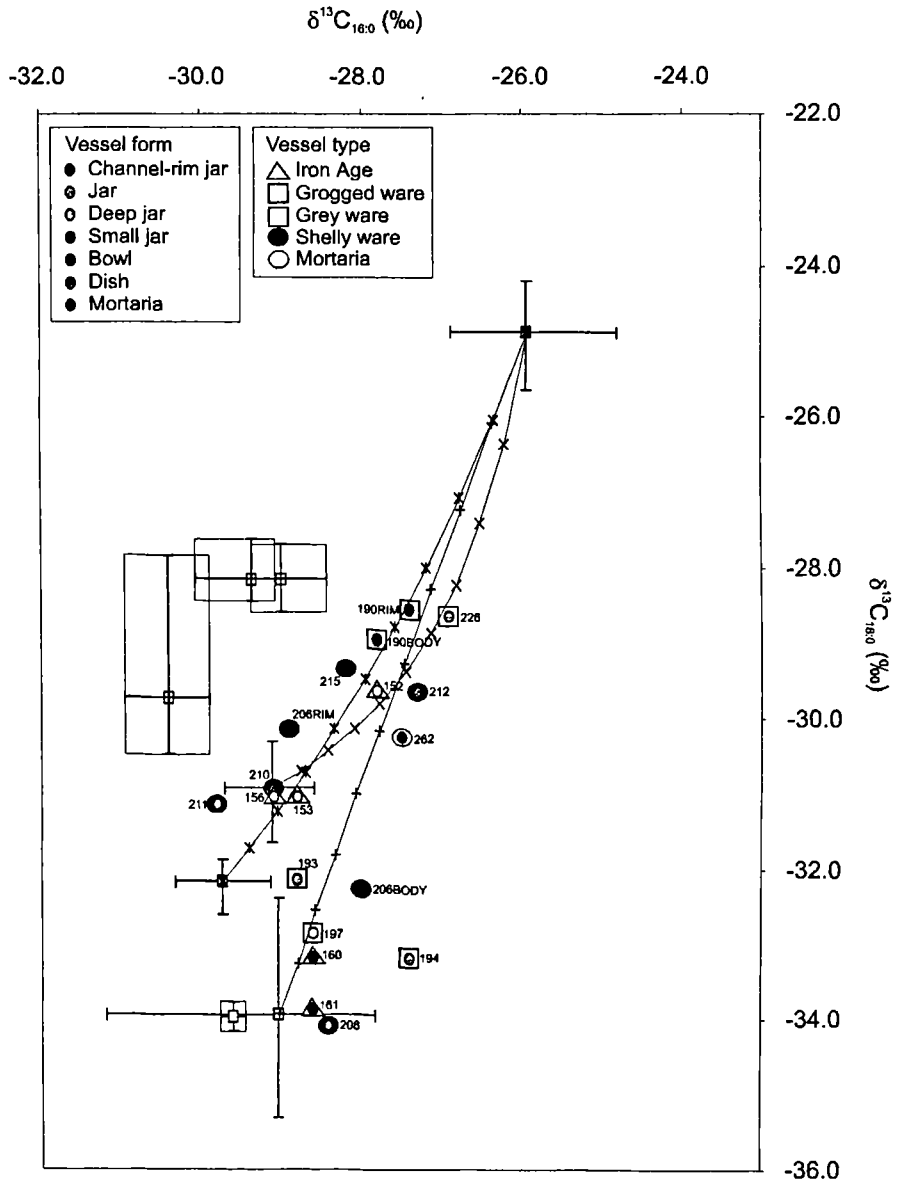


Figure 6.9 Plot of the $\delta^{13}C$ values of the major *n*-alkanoic acid components ($C_{16:0}$ and $C_{18:0}$) from the lipid extracts of potsherds from the Iron Age/Romano-British site of Stanwick, Northamptonshire, compared with vessel type and form.

6.4.2 *Sites with an unusually strong bias in the faunal record*

6.4.2.1 Wicken Bonhunt (Romano-British/Middle Saxon)

The aim of these analyses was to investigate whether the high proportion of pig bone present at the site was reflected in residues preserved in the potsherds. The $\delta^{13}C$ values obtained for the potsherd extracts from the Middle Saxon site plot in a broad distribution between the modern ruminant and non-ruminant reference fats (Fig. 6.10; Table 7, Appendix 6, p. 391). The fatty acids from the archaeological extracts are more depleted than those in the modern reference pig fats by up to 6‰. Several of the remnant fats plot close to the reference sheep adipose, although the majority of archaeological fats plot

along the line of the mixing curve, indicating that these data may represent mixtures of fats processed in the same vessel or multiple usage of vessels. There is no clear correlation between the archaeological data and the porcine reference fats, nor is there any indication from the stable carbon isotope data that any of the archaeological fats from Wicken Bonhunt derive from a dairy origin.

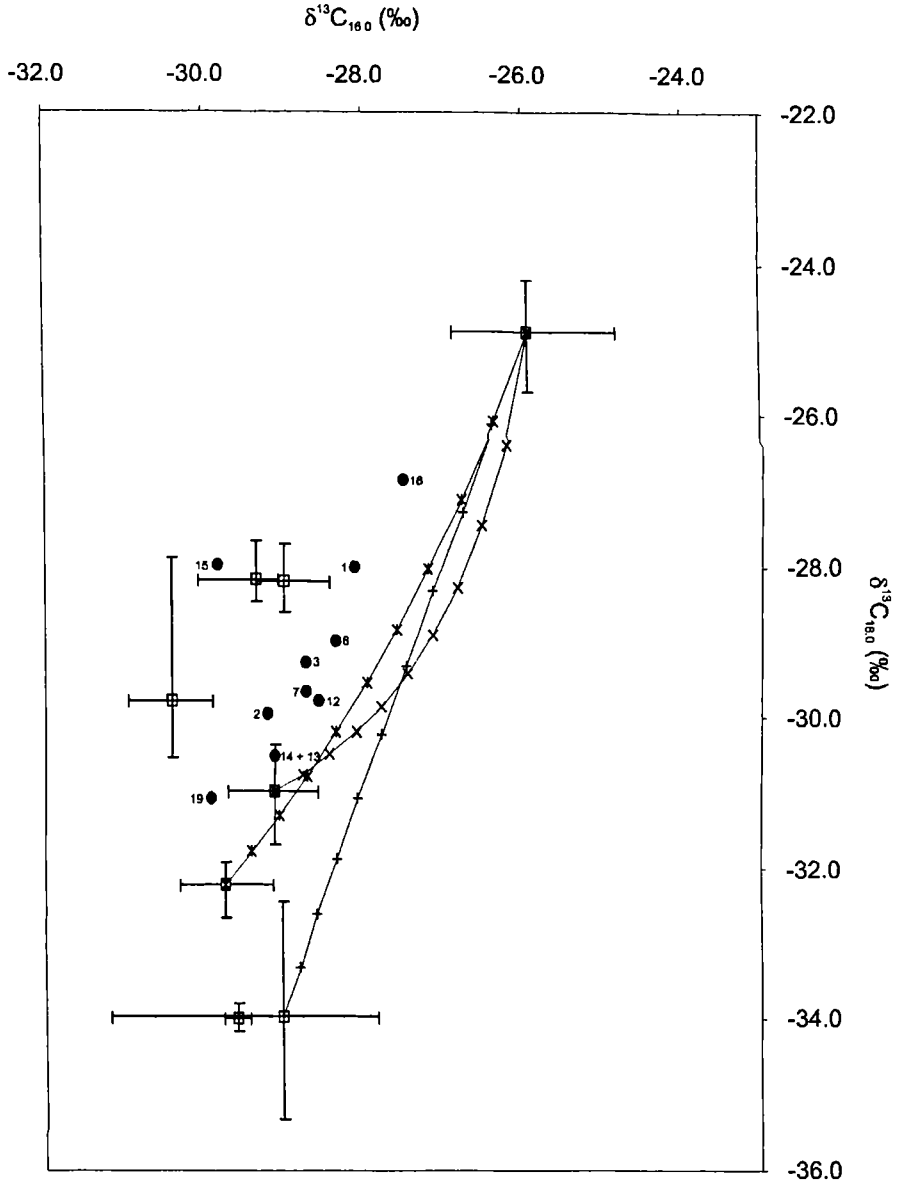


Figure 6.10 Plot of the $\delta^{13}C$ values of the $C_{16:0}$ and $C_{18:0}$ fatty acids in solvent extracts of Wicken Bonhunt potsherds compared with data from modern reference fats.

6.4.2.2 Botai (early Neolithic)

Potsherds from Botai were sampled in anticipation of retrieving data from degraded horse fats due to the strongly attested association of this site with horse breeding. The $\delta^{13}C$ values obtained for fatty acids in the potsherd extracts are shown in Figure 6.11 (Table 8,

Appendix 6, p. 391). The data points group together with mean $\delta^{13}C$ values of -27.1‰ for the $C_{16:0}$ and -27.5‰ for the $C_{18:0}$ fatty acids, but are distinct from the modern reference fats. The grouping of the data for the archaeological fats is relatively tight, indicating that these remnant fats all derive from the same animal origin. The remnant fats are less depleted by approximately 2-3‰ (in both the $C_{16:0}$ and $C_{18:0}$ fatty acids) than the modern reference horse fats from the UK. However, this difference can be attributed to differences in the isotopic composition of the diet of the horses raised in Kazakhstan from that of modern horses raised on forage in the UK. The data indicate that comparison of the stable isotope data from the fats of animals raised in different geographic locations are not directly comparable.

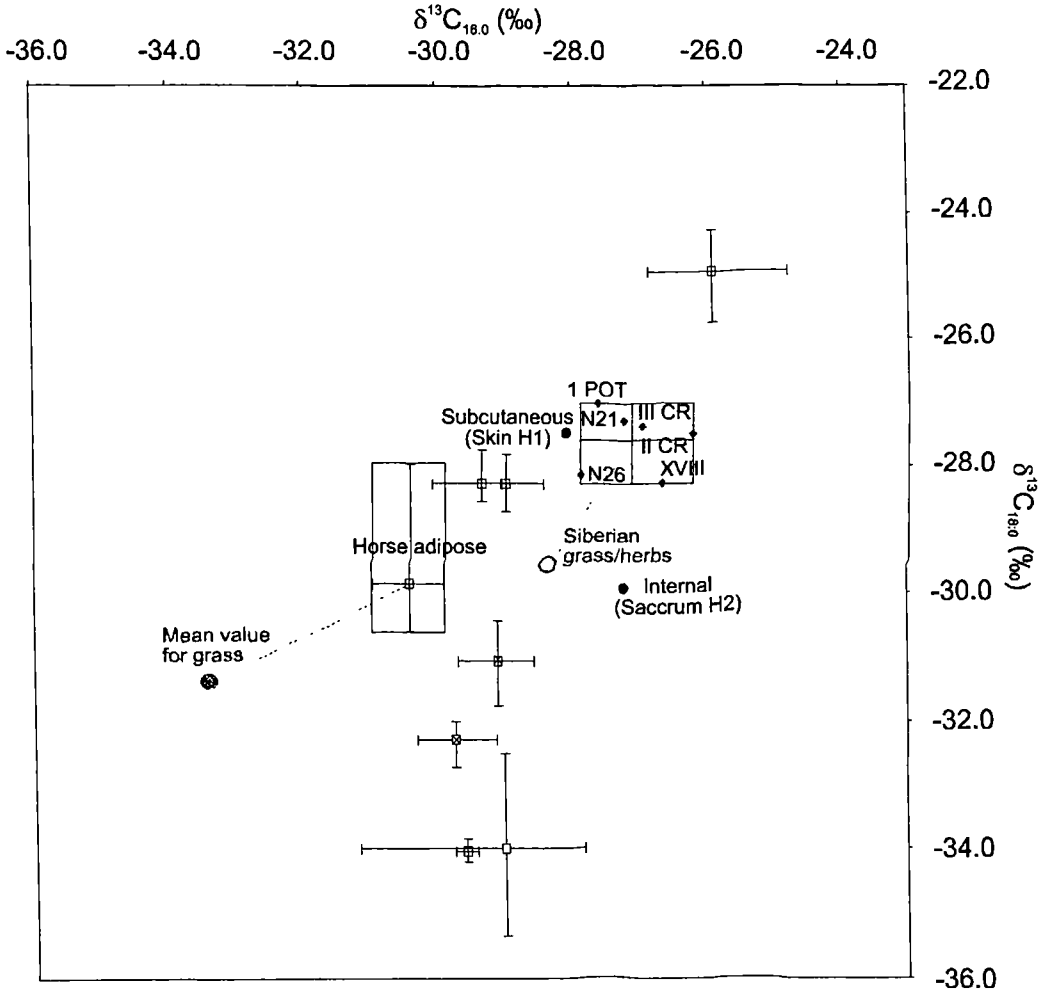


Figure 6.11 Plot of the $\delta^{13}C$ values of the $C_{16:0}$ and $C_{18:0}$ fatty acids in extracts of archaeological potsherds from Botai, Kazakhstan, compared with data for reference animal fats and the fatty acid components in their diet.

6.4.3 Prehistoric archaeological sites

6.4.3.1 Yarnton Cresswell field (early-middle Iron Age)

The stable isotope data for the majority of the Yarnton Cresswell field extracts are relatively depleted in ^{13}C and plot in the region of the ruminant adipose and dairy fats (Fig. 6.12; Table 10, Appendix 6, p. 392).

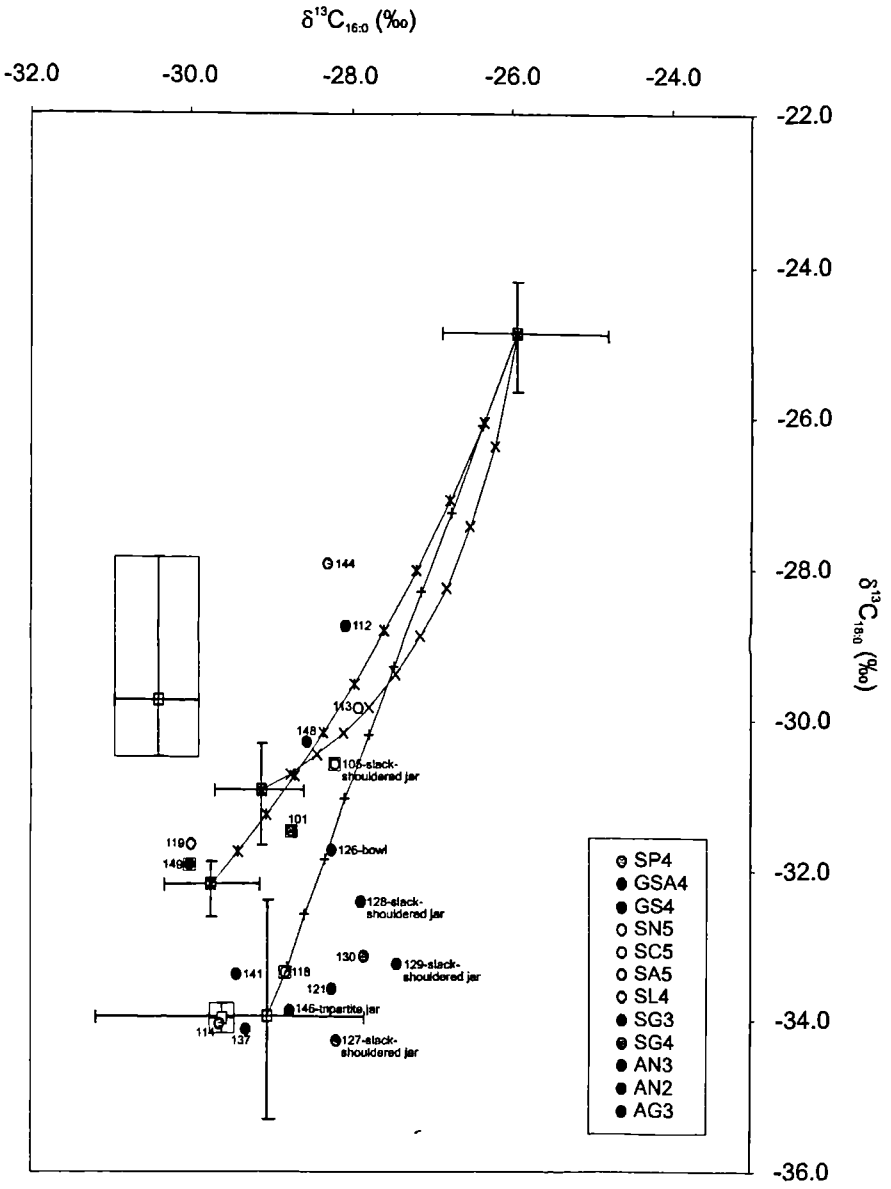


Figure 6.12 Plot of the $\delta^{13}C$ values of the fatty acids from lipid extracts of the Yarnton Cresswell field assemblage correlated with vessel fabric and form (where known) and compared with the data obtained for the modern reference fats. The fabric type abbreviations are explained fully in Table 7, Appendix 1 (pp. 346-347).

Approximately half of the archaeological extracts plot within the range of the modern reference cows' milk. Several extracts plot close to the mixing curves between the ruminant and non-ruminant reference fats. The extract from sample 144 is the least

depleted in ^{13}C. None of the remnant fats correlate with the reference porcine fats. There is some correlation between fabric types from Cresswell field and the $\delta^{13}C$ values of the extracts (Fig. 6.12), with the majority of the GSA4 (grog, shell and quartz sand; coarse textured) and SG3 (shell and grog; medium coarse textured) types plotting within the range for reference dairy fats as does sample 114 [fabric type AG3 (quartz sand and grog; medium-coarse textured)]. Sample 144 [fabric type SP4 (shell and clay pellets; coarse textured)] contains the remnant fat exhibiting the least depleted $\delta^{13}C$ values.

6.4.3.2 Yarnton flood plain (Neolithic-Bronze Age)

The stable carbon isotope data for fatty acids from the Yarnton flood plain extracts are plotted in Figure 6.13 (Table 11, Appendix 6, p. 392). The majority of the archaeological data points cluster in the region of the ruminant fats, with 4 samples plotting well within the range for the reference cows' milk fats. Only one data point (sample 38) out of 11 analysed falls within the range of the non-ruminant (e.g. porcine) reference fats. Several data points, including sample nos. 49, 23, 5 and 4 plot around the mixing curve between the milk and non-ruminant reference fats. In general the archaeological samples comprising higher abundances (>100 $\mu g\ g^{-1}$) of absorbed lipid also exhibited more depleted $\delta^{13}C$ values, resembling dairy fats. This is possibly a reflection of the ease with which certain fats are absorbed within the porous pottery, or the different ways in which vessels were used to process commodities, i.e. boiling or roasting. During the dosing of sherds for laboratory decay experiments (discussed in Chapter 7) we noted that substantially larger quantities of fat are absorbed when soaked in butter fat than in milk or adipose fats (Dudd, Aillaud and Evershed, unpublished data), suggesting that archaeological vessels containing substantial quantities of remnant dairy fats may have contained butter rather than milk fats.

Correlation of vessel type with $\delta^{13}C$ values reveals a distinction between the residues from the Peterborough ware and the Grooved ware vessels, with the former yielding $\delta^{13}C$ values comparable to the reference ruminant fats and the latter consistent with reference porcine fats. This result is significant since we have recognised the same distinction in residues from Peterborough and Grooved ware vessels from the Neolithic settlement at Upper Ninepence, Walton. Figure 6.13 also indicates that a range of different vessel types,

including Peterborough and Mortlake wares from the mid-late Neolithic, a beaker and an Early-Middle Bronze Age vessel, appear to have been associated with the processing of dairy fats.

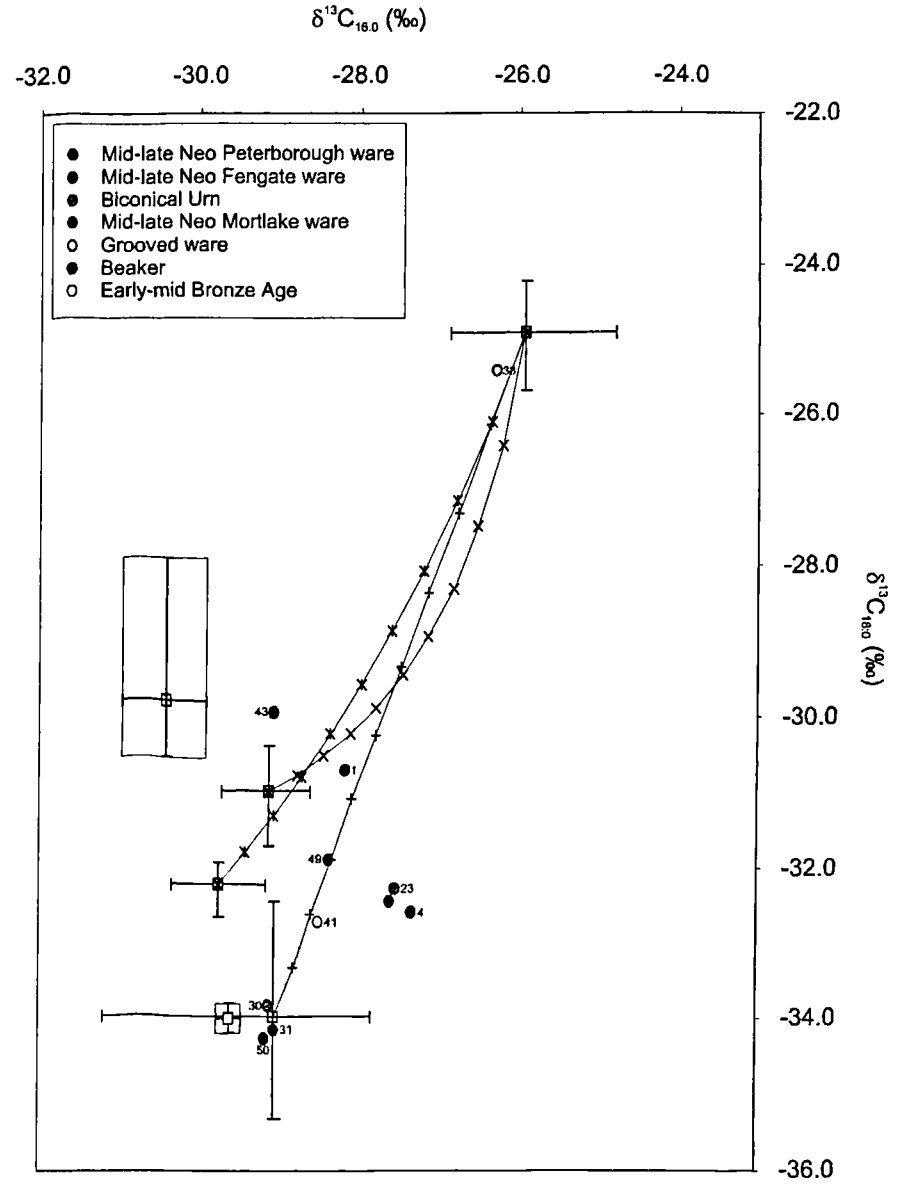


Figure 6.13 Plot of the $\delta^{13}C$ values of the fatty acids from lipid extracts of the Yarnton flood plain assemblage correlated with vessel type and compared with the data obtained for the modern reference fats typical of the archaeological period.

6.4.3.3 Eton Lake End Road (late Neolithic-Early Bronze Age)

Similar to the Eton Rowing Lake assemblage, the majority of the extracts from Eton Lake End Road plot within the region of the reference ruminant dairy fats, with $\delta^{13}C$ values of <-28‰ for the $C_{16:0}$ fatty acid and <-32‰ for the $C_{18:0}$ fatty acid (Fig. 6.14; Table 12, Appendix 6, p. 393). Three of the extracts, NRA 8-2164, NRA 2-rim and NRA 1 are slightly less depleted in ^{13}C, particularly in the $C_{18:0}$ fatty acid, and cluster with the reference ruminant adipose fats. Only one sample, NRA 4 is less depleted still and falls along the mixing curve between the ranges of the ruminant adipose and non-ruminant fats, possibly representing a mixture of ruminant and non-ruminant fats.

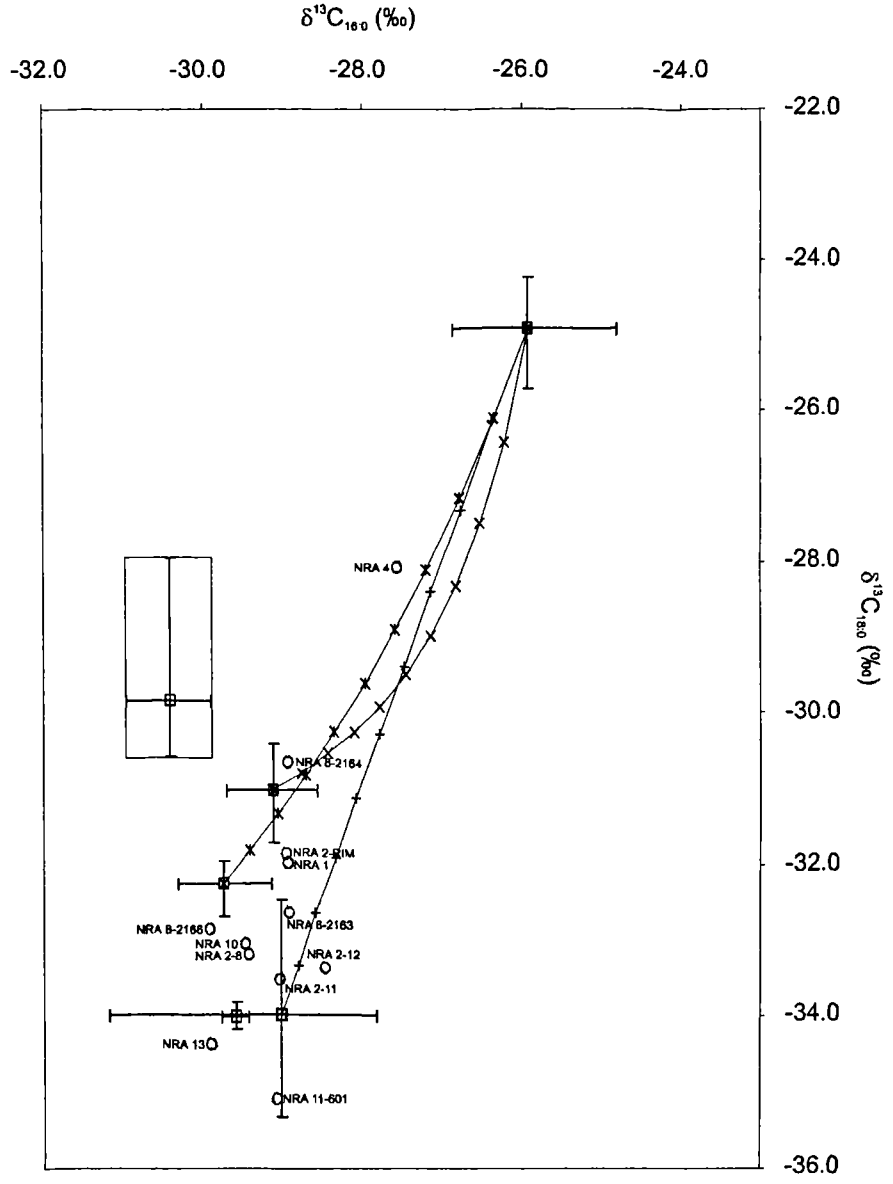


Figure 6.14 Plot of the $\delta^{13}C$ values of the fatty acids from lipid extracts of the Eton Lake End Road assemblage compared with the data obtained for the modern reference fats.

6.4.3.4 Eton Rowing Lake (early Neolithic)

All of the stable isotope data for the Eton Rowing Lake samples cluster within the ranges of the reference ruminant adipose and dairy fats (Fig. 6.15; Table 13, Appendix 6, p. 393), indicating that all of the remnant fats are derived from a ruminant source. Samples 12 and 20 are less depleted than the other samples and correlate with the data for the reference adipose fats, while the remainder correlate well with the reference dairy fats. None of the extracts have $\delta^{13}C$ values consistent with a non-ruminant origin.

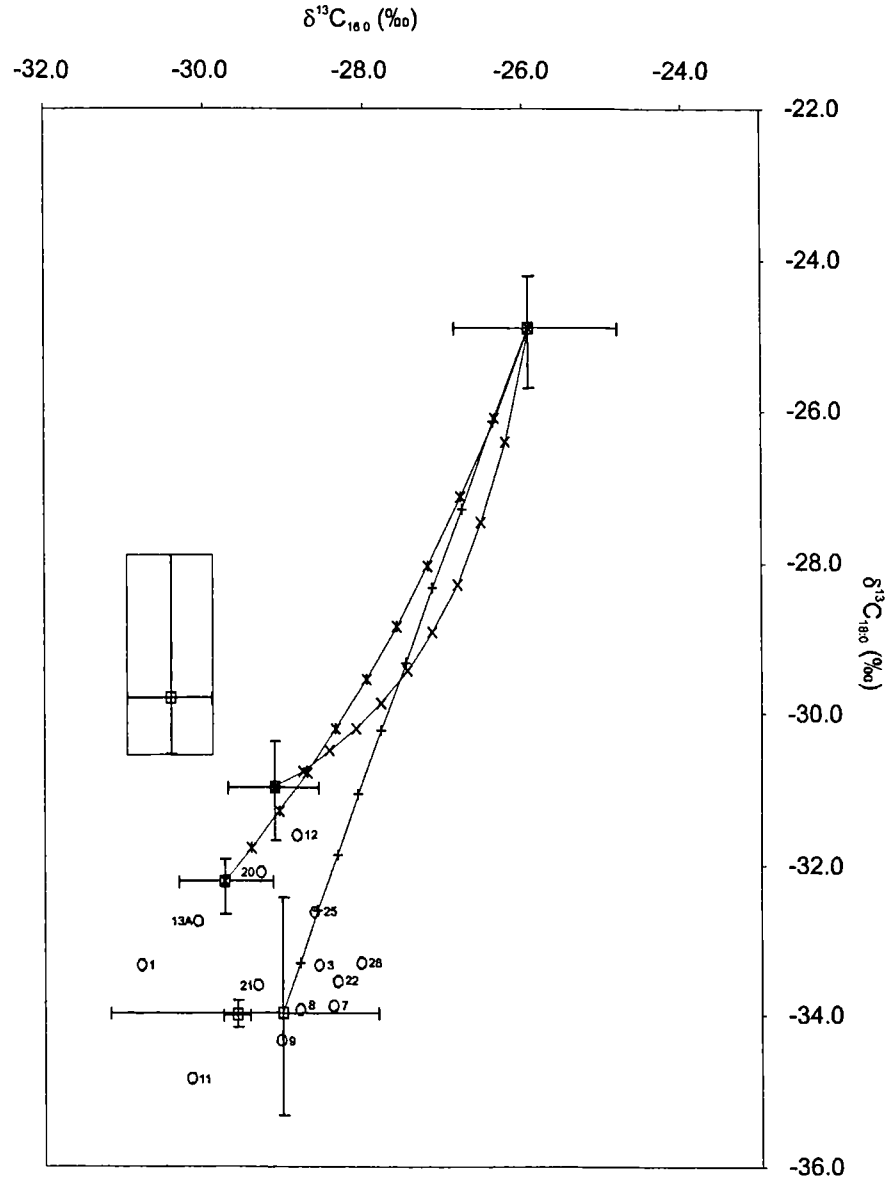


Figure 6.15 Plot of the $\delta^{13}C$ values of the fatty acids from lipid extracts of the Eton Rowing Lake assemblage compared with the data obtained for the modern reference fats typical of the archaeological period.

6.4.3.5 Upper Ninepence (early-late Neolithic)

The $\delta^{13}C$ values of the $C_{16:0}$ and $C_{18:0}$ fatty acids in three absorbed residues from the Peterborough ware (P1, P3 and P5), two absorbed residues from the Grooved ware (P66 and P68) and three carbonised (interior) surface residues from the Grooved ware (P33, P38 and P39) are plotted in Figure 6.16 (Table 14, Appendix 6, p. 394). Clearly, there is a distinction between the absorbed residues from the Peterborough ware and the Grooved ware and between the absorbed and carbonised residues from the Grooved ware, based on differences in the $\delta^{13}C$ values of both the $C_{16:0}$ and $C_{18:0}$ fatty acids

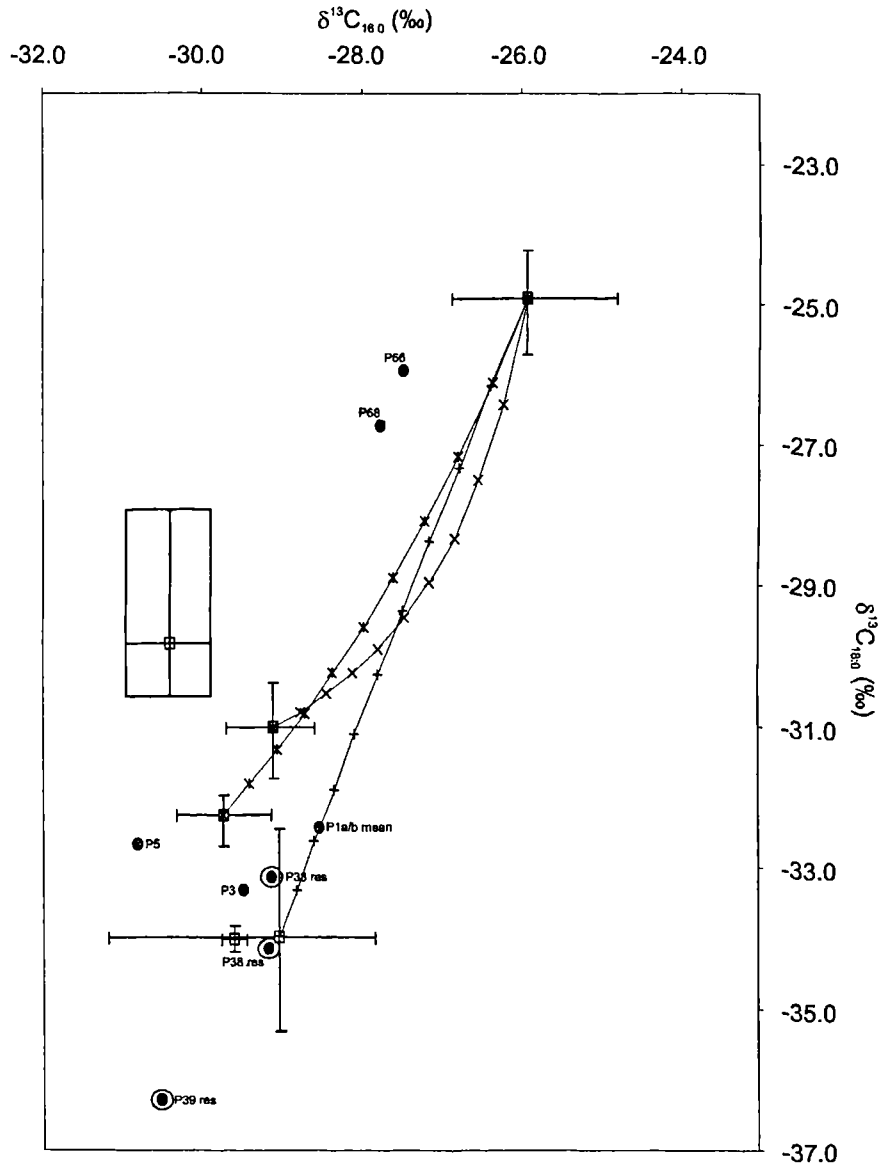


Figure 6.16 Plot of the $\delta^{13}C$ values for the major *n*-alkanoic acid ($C_{16:0}$ and $C_{18:0}$) components of the lipid extracts of potsherds from the Walton assemblage: Grooved Ware = blue-filled circles; Peterborough Ware = green-filled circles; Carbonised surface residues = yellow ring around the data point.

The absorbed archaeological fats from the Grooved ware (both from site context 133) plot together near to the non-ruminant (e.g. porcine) reference fats, whilst the three archaeological fats from the Peterborough ware plot in the region of the ruminant fats, within the range of the reference dairy fats. The carbonised residues adhering to three other Grooved ware vessels, all excavated from the same pit, plot with the reference dairy fats, with the exception of sample P39, which is more depleted in ^{13}C. The absorbed residues from these same vessels were poorly preserved, all comprising <13 $\mu g\ g^{-1}$ of lipid. The Grooved ware vessels associated with ruminant fat residues were excavated from a different archaeological feature than those of the same period corresponding with the non-ruminant reference fats.

6.4.4 Siberian horse tissues

Since the remnant fats from Botai were not directly comparable with UK reference horse fats, we obtained samples of Siberian horse fats for use as reference data. The $\delta^{13}C$ values obtained for the $C_{16:0}$ and $C_{18:0}$ fatty acids in the archaeological horse fats from the Siberian tomb are less depleted than the modern reference horse fats, particularly with respect to the $C_{18:0}$ fatty acid (Fig. 6.11; Table 9, Appendix 6, p. 391). The $\delta^{13}C$ values of the fatty acids from the internal (sacrum) and subcutaneous (skin) fat samples from the Siberian horses vary by ca. 1‰ and 2.4‰ for the $C_{16:0}$ and $C_{18:0}$, respectively. This may reflect the fact that the fat sample from the sacrum resembled adipocere, consisting predominantly of free fatty acids, which may have been contaminated by fatty acids from micro-organisms. There may also be some natural variation between tissues from different parts of the body.

The bulk $\delta^{13}C$ values obtained for the grass in the stomach of the Siberian horse were more depleted by approximately 2‰ than modern UK grasses (25.9‰ compared with 27.9‰); this was also reflected in the $\delta^{13}C$ values of the individual fatty acids in the grass (Fig. 6.11). The stomachs of these horses were found to contain a wide range of herbs and grasses typical of a rich upland pasture, whereas the diets of our modern reference horses was dominated by one or two grass species, from heavily grazed fields. Since non-ruminants and pseudo-ruminants are believed to be more directly influenced by their diet, the differences in the $\delta^{13}C$ values of their tissues can be readily related to differences in the composition of the diet. Clearly, the heavier dietary carbon consumed by the Siberian

horses had led to less depleted values for the fatty acids in their depot fats compared with our modern reference horses.

The data from the Siberian horse fats are clearly more comparable with the data obtained for the remnant fats from the Botai potsherd extracts than the modern horse fats. This positive correlation obtained for remnant fats from the a similar geographical region indicates that the absorbed residues in the Botai potsherds are, indeed, derived from horse fats processed in the vessels in antiquity.

6.5 Discussion

6.5.1 Reference fats

The stable isotope data have enabled clear distinctions to be drawn between adipose fats from the major species of domesticated ruminants, non-ruminants and poultry, and furthermore, has shown that significant differences exist between the composition of adipose and milk fats from dairy animals based on $\delta^{13}C$ measurements of the major saturated fatty acids.

6.5.1.1 Inter-species variation

The $\delta^{13}C$ values obtained for the reference ruminant adipose fats are relatively similar between different individuals of the same animal species. The sheep adipose gave mean $\delta^{13}C$ values of -29.1‰ ± 0.6 and -31‰ ± 0.7 and the cow adipose gave mean values of -29.7‰ ± 0.6 and -32.2‰ ± 0.4 for the $C_{16:0}$ and $C_{18:0}$ fatty acids, respectively. The variation can be attributed to the way in which ruminants can break down and reassimilate components from various sources of carbon in the diet and also to the fact that body fats represent an average value for carbon accumulated over several months. Conversely, the $\delta^{13}C$ values measured for the bovine dairy fats cover a more broad range (mean values of -29.0‰ ± 2.2 and -34‰ ± 1.5 for the $C_{16:0}$ and $C_{18:0}$ fatty acids, respectively), which is thought to be partly a reflection of the recent diet of the animal, due to the turnover of carbon in milk production being significantly faster than that of body fats (Tieszen *et al.*, 1983). Thus the composition of the dairy fats may vary significantly in isotopic composition, e.g. according to the availability of particular forage materials or seasonal

variations in $\delta^{13}C$ values of the plant tissues. No correlation could be found between the stable isotope composition of the reference milk samples and the time of year or stage of lactation during which they were collected.

The range of $\delta^{13}C$ values measured for fatty acids in the porcine fats is similar but slightly greater than for the ruminant adipose fats and is probably representative of the range of foodstuffs a pig will consume and the direct routing of dietary fats to body fats. It is well established that in non-ruminants such as pigs, little modification of the fats occurs unless utilised for energy (Christie *et al.*, 1972). The range of $\delta^{13}C$ values for fatty acids in tissues of goose and chicken fats were comparable with the ruminant reference fats, however, the data for the same fatty acids in deer adipose fats varied significantly, by up to 2‰ and 4‰ in the $C_{16:0}$ and $C_{18:0}$ fatty acids, respectively. Since the deer fats were all taken from animals of the same herd raised on the same unimproved pasture, the data are surprisingly variable. To some extent this may be a reflection of 'ecological variability', referred to as the 0.2-2‰ standard deviation found for animals of the same species raised in similar environments on the same diets (DeNiro and Epstein, 1978; Teeri and Schoeller, 1979; Tieszen *et al.*, 1983).

6.5.1.2 Contribution of dietary fat to milk

It has been recognised that the $\delta^{13}C$ value of the $C_{18:0}$ component of milk obtained from cows grazing on C_3 pastures differs isotopically from the subcutaneous adipose fat of cattle grazing on the same pasture. This distinction reflects the well-established pathways involved in the formation of milk and adipose fats in ruminant animals (Church, 1988; McDonald *et al.*, 1988), demonstrated by Tove and Mochrie (1963) in an investigation of tissue and milk fats sampled simultaneously from cows fed whole ground soybeans. They observed that the levels of both $C_{18:0}$ and $C_{18:1}$ increased markedly in the milk fat. This was compensated for by a decrease in the percentage of $C_{14:0}$ and $C_{16:0}$, and confirmed the contribution of dietary long-chain fatty acids to milk fat in ruminant animals.

Figure 6.17 illustrates the probable contribution of carbon from different sources to milk lipids according to Dimick *et al.* (1970). The substantial proportions of short- and medium-chain fatty acids in milk lipids are a result of a very active *de novo* synthesis from the

simple metabolites acetate and β-hydroxybutyrate, which are supplied to the mammary gland (Dils, 1983). It is well-established that a proportion of the $C_{16:0}$ and essentially all the C_{18} acids are derived *via* the circulating blood lipids. $C_{16:0}$ is known to be derived from two sources; pre-formed from the blood, and synthesised within the mammary gland from 2 carbon units (Dimick *et al.*, 1970). Triacylglycerols in the blood may arise either directly from absorbed (exogenous) fat or from endogenous fat *via* liver synthesis of very low density lipoproteins (VLDL; Dils, 1983).

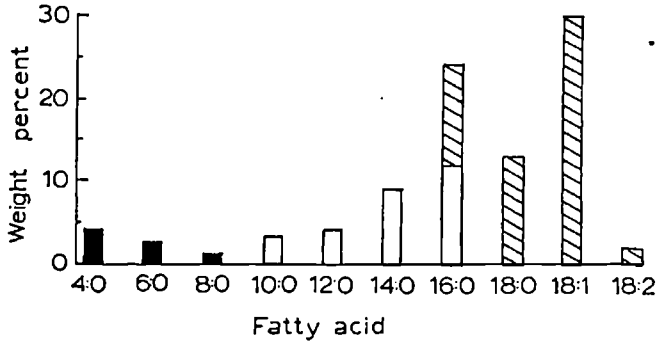


Figure 6.17 Probable origin of fatty acids in ruminant milk (from Dimick *et al.*, 1970): 4-carbon unit ■; malonyl-CoA pathway □ and circulating blood lipids ▨.

Various studies have been carried out to determine the proportions of dietary fat which contribute directly to fatty acids used in milk production. Banks *et al.* (1976a) showed that a low fat ration limited milk production, and tracer studies have indicated that 54% of dietary C_{18} fatty acids (Banks *et al.*, 1976b) and 76% of dietary $C_{18:2}$ (Palmquist and Mattos, 1978) are transferred directly to milk fat. However, estimates are dependant upon the physiological state of the animal and will also reflect changes in the contribution of endogenous (adipose) fatty acids to milk secretion as fatty acid intake varies, and contributions from rumen-synthesised fatty acids. Other isotopic labelling studies have estimated that 44% of milk fat is of direct dietary origin with approximately 6% of long-chain fatty acids from endogenous sources (Garton, 1963). Plowman *et al.* (1972) noted a rapid change in milk fatty acid composition when protected fat was fed to lactating ruminants; milk fat with increased polyunsaturated fatty acids was produced by feeding cows a diet containing a H_2CO-treated safflower oil-casein particle. The treatment

protected the $C_{18:2}$ acid in safflower oil from biohydrogenation in the rumen and $C_{18:2}$ acid content in the milk increased from 3 to 35% of the total fatty acids.

It has been suggested that in the mammary gland, long-chain fatty acids are produced by chain elongation. This has been demonstrated in ruminant adipose tissues by the formation of labelled $C_{18:0}$ and $C_{18:1}$ from [1-^{14}C]-acetate *in vitro*, where between 45-55% and 60-70% of the fatty acids synthesised in bovine (Pothoven *et al.*, 1974) and ovine (Deeth and Christie, 1979) adipose tissue slices, respectively, were elongated to C_{18} fatty acids. The $C_{18:0}$ in milk fat could therefore be partially derived from the $C_{14:0}$ and $C_{16:0}$ fatty acids in the diet following chain elongation, and would thus incorporate the relatively depleted carbon from these fatty acids. It should also be remembered that many other forage materials, including herbs and shrub vegetation, will also contribute long-chain fatty acids to the diet. As previously mentioned, these fatty acids are believed to be significantly more depleted than those in grass and probably contribute to the more depleted values for the $C_{18:0}$ in milk fat. Figure 6.18 shows the relative abundances of the individual long-chain fatty acids in a typical ruminant diet (mainly grass). The $C_{18:0}$ component comprises only 5% of the total, while together the unsaturated C_{18} fatty acids with $\delta^{13}C$ values of less than -34‰ constitute a total of 29%.

The stable isotope data obtained for the $C_{14:0}$, $C_{16:0}$ and $C_{18:0}$ fatty acids in the reference ruminant milk fats are also compared in Figure 6.18. There are large differences between the $\delta^{13}C$ values of the major fatty acid components of the milk fats, amounting to approximately 6‰ and 8‰ between the $C_{14:0}$ and $C_{18:0}$ in cow's milk and sheep milk, respectively. The $\delta^{13}C$ value of the $C_{14:0}$ in milk is clearly not consistent with a direct dietary origin, due to the difference in $\delta^{13}C$ value from the $C_{14:0}$ in the grass, but reflects the value for the bulk diet (i.e. mainly carbohydrate). The $\delta^{13}C$ value of the $C_{18:0}$ fatty acid in the milk fats does not reflect the bulk value for milk which indicates that this component is derived, at least partially, from a source other than the carbohydrate in the diet. The relatively depleted $\delta^{13}C$ data recorded for the C_{16} and C_{18} fatty acids in the diet (ca. -27 to -37‰) indicate that these components could be contributing to the depleted values of the C_{16} and C_{18} fatty acids in the milk fat.

The almost linear relationship between the $C_{14:0}$, $C_{16:0}$ and $C_{18:0}$ fatty acids shown in Figure 6.18 supports the work by Dimick *et al.* (1970; Fig. 6.17) who have suggested that the $C_{14:0}$ fatty acid is derived predominantly from the malonyl-CoA pathway while the $C_{16:0}$ fatty acid forms from approximately equal contributions from both the circulating blood lipids (including dietary fatty acids) and *de novo* synthesis. The majority of the C_{18} is reported to be derived from circulating blood lipids which include up to 50% of long-chain dietary fatty acids ($C_{18:0}$ and unsaturated C_{18} after biohydrogenation).

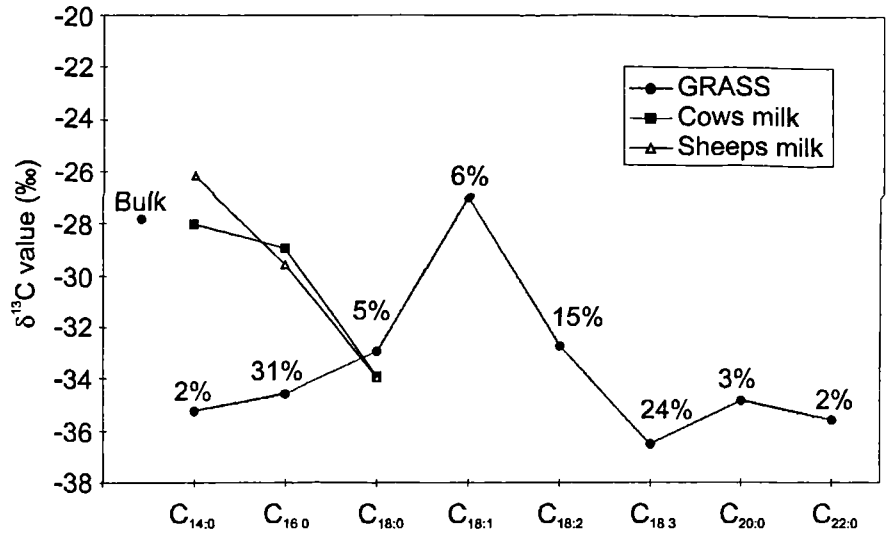


Figure 6.18 Stable carbon isotope data obtained for the major saturated fatty acids in cows (mean of 8 individuals) and sheep (mean of 2 individuals) milk compared with $\delta^{13}C$ values for the bulk diet (grass) and individual fatty acids in the diet. The relative abundances (mean %) of the different fatty acids in grass are shown.

Stable carbon isotope analyses of the different biochemical fractions which comprise grasses and herbs from unimproved pastures are currently under investigation in our laboratory in order to investigate the various sources of carbon available to animals which consume them. Results obtained to date have shown C_{18} fatty acids with highly depleted $\delta^{13}C$ values in the range of -36‰, e.g. in herbs such as clover (G. Docherty, pers. comm.). The consumption of these highly depleted fatty acids would explain the more depleted values for the $C_{18:0}$ components in milk fat since herbivorous grazers will consume a variety of foliage and herbage as well as grass. The regulation of milk production and adipose fat formation is far from simple and as yet not fully understood but since fatty acid output in the milk of lactating cows usually exceeds daily intake of fatty acid, lipid

metabolism must play an important, if not central, role in the energy economy of the lactating cow.

In addition to dietary fat content and metabolic variations, the range of stable carbon isotope values obtained for the reference and archaeological milk samples probably reflect variation in the proportion of fibre in the animals diets, the effects of a range of environmental stresses on the animals and also the stage of lactation. Palmquist and Mattos (1978) suggest that their estimates of fatty acid transfer from the diet may not be valid during the non-steady state when the cow is rapidly losing adipose stores during early lactation.

Changes in plant carbon isotope ratios may occur due to environmental heterogeneity which are most likely associated with either large differences in soil moisture content (affecting plant water status) or light intensity (Fogel and Cifuentes, 1993; Lockheart *et al.*, 1997). Lowdon and Dyck (1974) have shown that the $\delta^{13}C$ values of maple leaves and a grass species collected at a single location may vary more than 5‰ during the growing season. A recent study of the $\delta^{13}C$ values of individual fatty acids in vegetable oils has shown sources of variability relating to geographical origin of the oil, year of harvest and the particular variety of the oil (Woodbury *et al.*, 1998).

6.5.2 Archaeological data

All of the archaeological fat extracts prepared as FAME and analysed by GC-C-IRMS have yielded $\delta^{13}C$ values which correlate closely with the range of data obtained for the modern reference fats. The data from each site appear to correspond either with the ruminant dairy, ruminant adipose, non-ruminant adipose or lie along the line of the theoretical mixing curve between ruminant and non-ruminant adipose fats. None of the values appear to be erroneous or affected adversely by decay so that they lie far from the reference fat data points. Coupled with the distributional data, which show that the fats comprise an abundance of saturated $C_{16:0}$ and $C_{18:0}$ fatty acids characteristic of animal fats, the isotopic data have also indicated that all the archaeological fats studied derive from a terrestrial source rather than a marine source.

The study of assemblages from the well-documented sites of West Cotton and Stanwick has provided an excellent starting point for the exploratory use of compound specific $\delta^{13}C$ values in the identification of remnant fats, since the major domesticated animals at these sites are known to be predominantly of ovine, bovine or porcine origin, enabling assumptions to be made about the remnant fats processed in the vessels. The data from West Cotton have shown how stable carbon isotope measurements can be used to distinguish between remnant fats which derive from non-ruminant, ruminant adipose and ruminant dairy fat origins. A number of remnant fats plotted along the mixing lines between the reference ruminant and the reference non-ruminant fats. These are thought to represent adipose fats, the intermediate $\delta^{13}C$ values derived from the mixing of different fats, the non-specific use of individual vessels in processing animal products or possibly shifted isotopic values due to a different dietary regime in antiquity. The data for the Stanwick extracts exhibit a comparable spread of $\delta^{13}C$ values for the ruminant fats, however, no non-ruminant (e.g. porcine) fats are present.

The $\delta^{13}C$ analysis of the Saxon pottery residues from Wicken Bonhunt has not provided clear evidence for the processing of pigs in these vessels, however, based on comparison of the data with the theoretical mixing curves we have tentatively identified mixtures of porcine and ruminant fats. The apparent lack of remnant porcine fats may be a reflection of cooking methods, since pigs are traditionally thought to have been cooked by spit-roasting, perhaps with processing in pottery vessels of secondary importance. Furthermore, since faunal evidence of other animal species have also been recovered from the site, it is likely that these species are also represented in domestic wares with the more depleted data representing fats from the ruminant species identified amongst the faunal remains. The emphasis at this site clearly appears to be on the production and processing of animal meat/fat in preference to dairy products.

The ranges of stable carbon isotope data for the prehistoric assemblages from Yarnton flood plain and Cresswell field assemblages are comparable, with the majority of residues from both sites corresponding to the reference ruminant fats. At both sites there are data points for the archaeological samples which plot just outside the range for the reference cows' milk fat. These residues would appear to represent degraded dairy fats or mixtures of

dairy fats and non-ruminant fats, as would arise in multiple vessel use (discussed by Charters, 1996). In the earlier, Neolithic assemblage, one residue corresponds to the reference non-ruminant fats. In the later period, there are a number of residues which would appear to represent mixtures of ruminant and non-ruminant fats. Similarly, at Eton Rowing Lake, the early Neolithic assemblage contains residues which cluster within the region of the ruminant fats, whilst the Neolithic/Bronze Age sherds from the nearby Lake End Road site also contain residues which are less depleted isotopically and may represent more varied vessel use. Stable carbon isotope data from the prehistoric assemblage from Walton have shown clear differences exist between remnant fats from the different phases of the site, associated with the different pottery traditions, with ruminant fats, possibly from a dairy origin, identified in both the early and late Neolithic periods, but non-ruminant (e.g. porcine) fats only present in the later Grooved ware assemblage.

The analysis of materials from Siberia and Kazakhstan have illustrated that caution needs to be taken when comparing isotopic data from samples originating from different geographical locations, probably largely due to the variation in the stable carbon isotope composition of dietary components, e.g. grass and forage, rather than differences in metabolism or physiology of different breeds of horse. Nonetheless, the stable carbon isotope data have clearly indicated that the Botai potsherd residues derive from horse fats due to the close correlation between the Siberian horse fats and the archaeological pot residues. These data provide direct evidence for the exploitation of horses for their meat as well as for work animals by the Kazakhstan peoples.

6.6 Conclusions

Based on the stable carbon isotope analyses carried out on the modern reference animal fats and archaeological fats, the following conclusions can be drawn:

1. Stable carbon isotopic analysis has enabled distinctions to be drawn between modern fats from the major domesticated ruminant and non-ruminant animal species. The less depleted $\delta^{13}C$ values seen for the fatty acids in non-ruminant fats compared to the fatty acids in ruminant fats reflect differences in the complex metabolic and biochemical

processes involved in the formation of body fats between the different species and to a lesser extent reflect differences in diet. These distinctions are clearly reflected in the archaeological fats from West Cotton.

2. Fats from a number of archaeological sites have been tentatively identified as deriving from a ruminant dairy origin based upon the greater depletion in the $\delta^{13}C$ values of $C_{18:0}$ fatty acids in dairy fats compared with adipose fats. It is proposed that the difference in the isotopic signal of the $C_{18:0}$ fatty acid in milk and adipose derives largely from the established metabolic pathways involved in lactation, the physiological demands of which result in a shift in the energy balance such that a greater proportion of the $C_{18:0}$ fatty acid present in milk is derived directly from the long-chain fatty acids in the diet. The $C_{18:0}$ fatty acid is produced partially through biohydrogenation in the rumen, therefore reflecting the depleted $\delta^{13}C$ values of the $C_{18:1}$, $C_{18:2}$ and $C_{18:3}$ fatty acids which predominate in grass and forage materials, and partially through chain elongation of the $C_{14:0}$ and $C_{16:0}$ fatty acids in the diet. The more negative $\delta^{13}C$ values (ca. -32.5 to -34.0‰) seen for $C_{18:0}$ in milk compares favourably with the depleted values recorded for C_{18} fatty acids in pastures and fodders, i.e. up to -36.5‰. These distinctions are clearly reflected in the archaeological fats from West Cotton and Stanwick.

3. It is a well recognised fact that $\delta^{13}C$ values of fatty acids of plants will always be more depleted, by approximately 5‰, than those of carbohydrates from the same source (Deines, 1980). Thus, notwithstanding the proportion of carbon routed from stored fat and dietary carbohydrate, milk and adipose fats from animals raised on similar diets are separable since the isotopic relationships between the major biochemical fractions, in this case milk and adipose fats, will always be qualitatively preserved, thus establishing a secure basis for detecting dairying at different geographical locations and during different periods in prehistory.

4. The isotopic data have provided the first direct evidence for the processing of dairy fats at prehistoric sites, and has indicated that a large number of vessels from both Yarnton and Eton had been used for the storage or processing of milk or milk products. The fatty acids recovered from the vessels exhibited highly depleted $\delta^{13}C$ values for the $C_{18:0}$

components which correlated closely with the dairy fats from modern animals raised on C_3 pastures.

If the trends in the isotopic data are supported by the other chemical criteria being considered, then the close correlation between the isotopic data obtained for the remnant fats and the modern fats is remarkable considering the age of some of the assemblages and thus the potential for alteration of the original isotopic signal. Clearly, based on the data presented herein, the measurement of stable carbon isotope ratios has proven the single most effective criterion of those considered in distinguishing between degraded fats of ruminant, non-ruminant and dairy origin. The reliability of the stable carbon isotope signal as an indicator of origin of degraded animal fats is the subject of further study in Chapter 7.

CHAPTER 7

Assessing Patterns of Decay and Microbial Lipid Contributions During Laboratory Degradations of Fats and Oils and Pure Triacylglycerols Absorbed in Ceramic Potsherds

7.1 Introduction

Although lipids have been recognised as surviving absorbed within the fabric of archaeological pottery vessels, *e.g.* cooking and storage jars, for more than twenty years (Condamin *et al.*, 1976; Condamin and Formenti, 1978; Rottländer, 1990; Heron and Evershed, 1993, and references therein), the extent of the information that can be recovered from such residues is only just beginning to be fully realised. For example, bound lipids have recently been identified within the matrix of pottery vessels from both waterlogged and arid burial environments using alkaline hydrolysis to release solvent-insoluble components by cleavage of ester linkages (Regert *et al.*, 1998). It is therefore increasingly important that we are able to correctly interpret the distributions and origins of components that we observe.

It is equally important to be able to recognise the distinction between lipid components which are derived from sources other than the original natural commodity deposited in the vessel. This may include post-excavation contamination, e.g. from conservation treatments, general handling, or due to the packaging of sherds in plastic bags or 'bubble-wrap'. The possibility of contaminants arising during wet chemical work-up is monitored by the routine analysis of 'blanks' during the extraction procedure. Although the surface of the sherds are cleaned with a modelling drill prior to extraction, the possibility of contamination arising from the burial environment has been considered previously by Heron *et al.* (1991), where lipid constituents from freshly excavated potsherds and burial soil adhering to the sherd surface were examined. Clear distinctions were drawn between the constituents in the soil and those absorbed into the sherds during use.

In addition, we should also consider the possible contribution from bacterial populations. It is almost inevitable that, during the period of discard and burial, bacterial action will alter the original distribution of the lipid components derived from vessel use and indeed, bacterial lipids (e.g. membrane components) are likely be deposited. It is well known that high molecular weight compounds forming storage or structural materials are readily transformed into assimilable components by fungal and bacterial enzymes (Killops and Killops, 1993). Since bacteria adapt and thrive readily where a suitable substrate is available (together with other essential nutrients), there is clearly a need to assess the effect

of bacterial contributions to the lipid components derived from the original archaeological commodities. Since we assume that degradation is partly bacterially-mediated we would expect lipid extracts to contain cellular components of the micro-organisms, or microbially reworked components, in addition to the lipid substrates initially present in the pottery. The most widely recognised markers of bacterial action are the branched-chain fatty acids of the *iso-* and *anteiso-* series which occur in many bacteria as the major acyl constituents of membrane lipids (Lösel, 1988; Schweizer, 1989).

So far within this study we have investigated the intrinsic chemical properties of animal fats in order to enable the origins of remnant fats to be reliably distinguished. However, of equal importance is the ability to recognise and understand the processes of decay which lead to the unique chemical signatures of remnant fats. In depth knowledge of the effects of hydrolysis, oxidation and polymerisation on lipid components is required to enable accurate interpretations of degraded lipid profiles and the identification of reliable biomarker components. Thus, in addition to using a range of analytical techniques to retrieve and identify components we have aimed to devise experiments to enable the decay of lipids in natural commodities to be monitored. It was anticipated that the results of such experiments would aid in our interpretations of lipid distributions in archaeological residues.

7.1.1 Preservation potential of lipids

Differences in the preservation potential of the various classes of organic components are significant in determining the nature of changes which will occur to a natural fat or oil during diagenesis in the burial environment. For example, Rottländer and Schlichtherle (1979) performed simulation experiments to demonstrate that oxidation of polyunsaturated fatty acids occurs more rapidly than oxidation of monounsaturated fatty acids. Short-chain components are preferentially removed by dissolution than their longer-chain counterparts (see Section 4.13) and phospholipids are known to be more labile than neutral lipids. Indeed in soil, phospholipids are believed to have only a transitory existence with the result that extractable phospholipids are assumed to derive from the living biomass. In comparative studies, it has been shown that proteins and carbohydrates are degraded at a much faster rate than either lipids or lignin (Meyers *et al.*, 1980; Benner *et al.*, 1984;

Hedges *et al.*, 1985). Other examples are listed by Logan *et al.* (1991) who gives an account of the factors affecting preservation of organic biomolecules and note the relatively high preservation potential of lipids compared with most other biochemical components in the geosphere, e.g. proteins and carbohydrates.

7.1.2 Hydrolysis

The decay of acyl lipids, e.g. triacylglycerols, phospholipids and wax esters, the major components in fresh animal fats and higher plant and fish oils, is thought to occur to a large extent through β-oxidation but is often initiated by hydrolysis. Ester hydrolysis is usually catalysed by acids or bases, although may also be catalysed by metal ions and enzymes (Satchell and Satchell, 1979), and in the case of the complete hydrolysis of triacylglycerols, results in the liberation of free glycerol and three free fatty acids (see Section 1.5). In contrast to other food constituents, such as carbohydrates and proteins, molecules of fats and oils possess relatively few reactive sites so that degradative reactions which take place are less varied than in the case of water-soluble components (Davídek *et al.*, 1990).

It is likely that a combination of factors, including the pH of ground waters and micro-organisms, will catalyse hydrolysis in the burial environment. In addition, fats and oils hydrolyse during processing and storage, passing to some extent from the bound form into their free form or *vice versa* (Davídek *et al.*, 1990), catalysed either by water at high temperatures or by natural, non-specific lipolytic enzymatic reactions. Mono- and diacylglycerols do not tend to accumulate because partially hydrolysed glycerol esters are more readily hydrolysed than intact triacylglycerols. Lipases are known to be particularly active at the lipid-water interface, and hence homogenisation and emulsification will tend to stimulate the enzymatic activity. Thus, the production of butter or cheese may result in the accelerated decay of dairy products compared to other animal fats. Milk lipases are largely destroyed during its pasteurisation so they are important only when raw milk is used for cheese making. Microbial enzymes are also known to catalyse the transesterification of triacylglycerols (Nielsen, 1985).

7.1.3 Oxidation

Oxidation reactions are probably the most common degradative reactions involved in the decay of food materials and are usually attributed to β-oxidation or to reaction of singlet oxygen with unsaturated lipid components to form hydroperoxides (Afanas'ev, 1987). Compounds containing both an aromatic and an alkyl or aliphatic moiety may undergo β-oxidation. The pathway of β-oxidation involves the step wise degradation of fatty acids (Fig. 7.1).

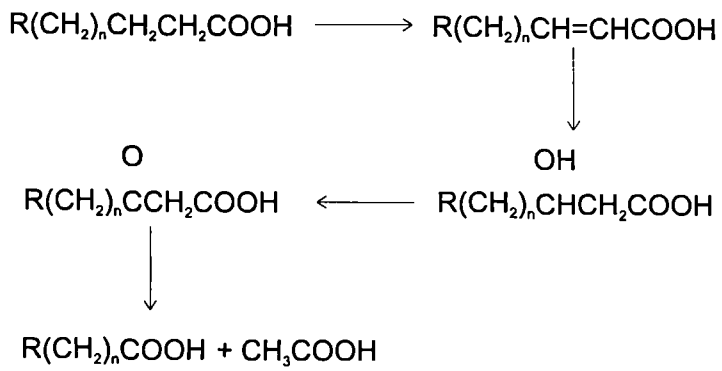


Figure 7.1 β-oxidation of alkanoic acids (Alexander, 1999).

The mechanism of hydroperoxide formation by reaction of singlet oxygen (photooxidation) with unsaturated free radical acids (Fig. 7.2) can occur *via* an intermediate peroxy radical which can abstract a hydrogen atom from another unsaturated acid and so propagate the chain reaction. However, this mechanism is only likely to effect lipid components adhering to the surface of vessels prior to burial since photooxidation is due to free radicals produced by ultraviolet light irradiation which catalyses the decomposition of oxygen complexes of fatty acids such as carbonyl compounds (RCOR) and hydroperoxides (ROOH). These mechanisms and others, including autoxidation and metal catalysed oxidation, have been widely reviewed (e.g. Davídek *et al.*, 1990; Frankel, 1998). In addition, microbial lipases, such as lipoxygenases, are likely to contribute to the decay of lipids in the burial environment, for example, by producing site-specific hydroperoxy derivatives of essential fatty acids, and microbially-mediated β-oxidation is known to occur under anaerobic conditions (Gurr and James, 1980).

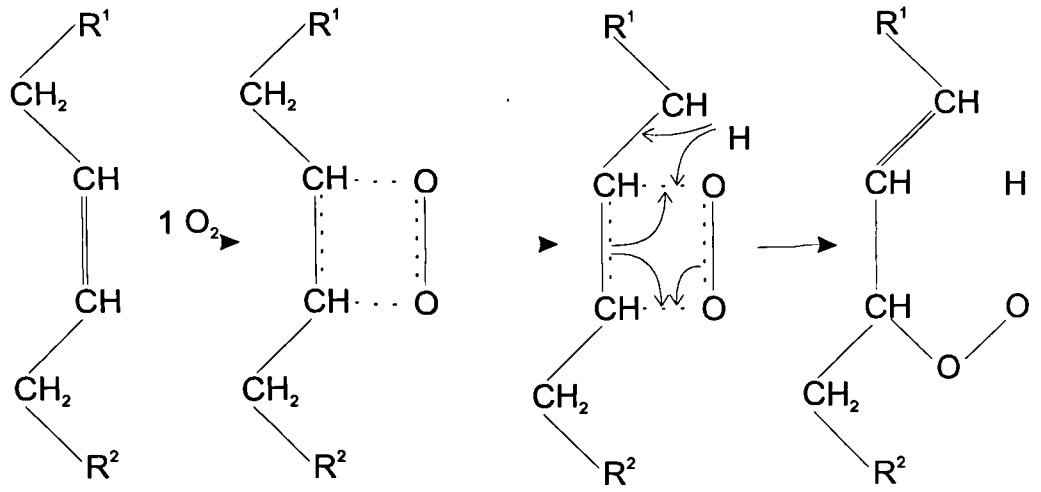


Figure 7.2 Reaction of singlet oxygen with the double bonds of unsaturated lipids to form hydroperoxides (from Afanas'ev, 1987).

The hydroperoxides are reactive species which can: (i) react further with singlet oxygen to produce hydroxyepidioxy and dioxolane derivatives; (ii) combine to give peroxy dimers by recombination of peroxy radicals; (iii) disproportionate into hydroxylic and oxodienoic derivatives; (iv) cleave to give low molecular weight aldehydes, alkenes, alcohols and alkanes, and (v) form covalent bonds with other food components to produce organic solvent-insoluble compounds.

Peroxidation of fatty acids is predominantly a chain reaction because of the high reactivity of peroxyl radicals with the weak allylic and bisallylic C-H bonds and the high reactivity of the resulting fatty acid radicals with oxygen. The weakest C-H bond in polyunsaturated fatty acids is in the bisallylic position and consequently this is the most reactive site for H-atom abstraction by free radicals, although other C-H bonds may be involved in abstraction (Simic *et al.*, 1992). Oxygen does not add to bisallylic radicals, however, due to interaction of the unpaired electron of the free radical with other atoms or groups within the radical which result in its reduced electron density. In oleic acid (*cis*-Δ^9-octadecenoic) the predominant sites of free radical attack are the allylic positions C_8 and C_{11}, while in linoleic acid (*cis*, *cis*-Δ^9, Δ^{12}-octadecadienoic) the most reactive site is the bisallylic position, C_{11}, and linolenic acid (*cis*, *cis*, *cis*-Δ^9, Δ^{12}, Δ^{15}-octadecatrienoic) has two reactive bisallylic sites, C_{11} and C_{14}. The hydroperoxides which result are labile and readily decompose, e.g. to unstable alkyl radicals which may react with surrounding fatty acids to generate alkanes

and alkyl radicals (e.g. Fig. 7.3). Passi *et al.* (1993) suggest that this oxidation pathway can lead to the formation of dicarboxylic acids, e.g. azelaic acid, by formation of the hydroperoxide at C_9 which is converted to the alkoxy radical and finally to the C_9 diacid *via* azelaic hemialdehyde. Whilst Passi *et al.* (1993) have shown that saturated short- and medium-chain length dicarboxylic acids are produced by oxidation of *cis*-polyunsaturated fatty acids, a full explanation of the mechanism by which they arise has yet to be provided. It is interesting to note, however, that diacids can be regarded as distinctive products of the oxidation of polyunsaturated fatty acids with particular double-bond positions. The long-chain mono- and dihydroxy acids are thought to be formed by the hydration of double-bonds.

We have recently identified the C_7 to C_{12} diacids and other lipid oxidation products, including ω-hydroxy acids and longer-chain hydroxy and dihydroxy acids (Fig. 7.4) covalently bound into solvent-insoluble residues entrapped within potsherds from waterlogged deposits (Regert *et al.*, 1998, in press; Frost, 1998). It is likely that these components were formed by extensive oxidation of the lipids in the commodities during processing. Only those components which became bound within the matrix of the pottery (e.g. through chemical bonding to the clay matrix or in polymeric matrices) are found preserved since oxidised moieties are often highly reactive and unlikely to survive as free lipids.

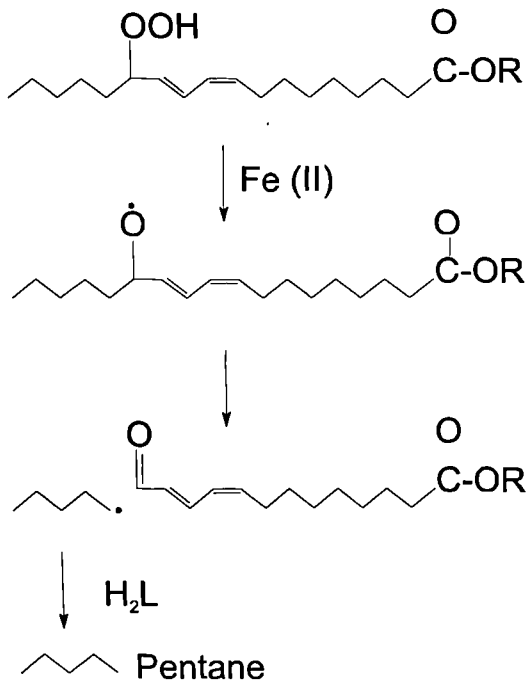


Figure 7.3 Formation of pentane from the 13-hydroperoxide of linoleic acid (H_2L; adapted from Simic and Taylor, 1987).

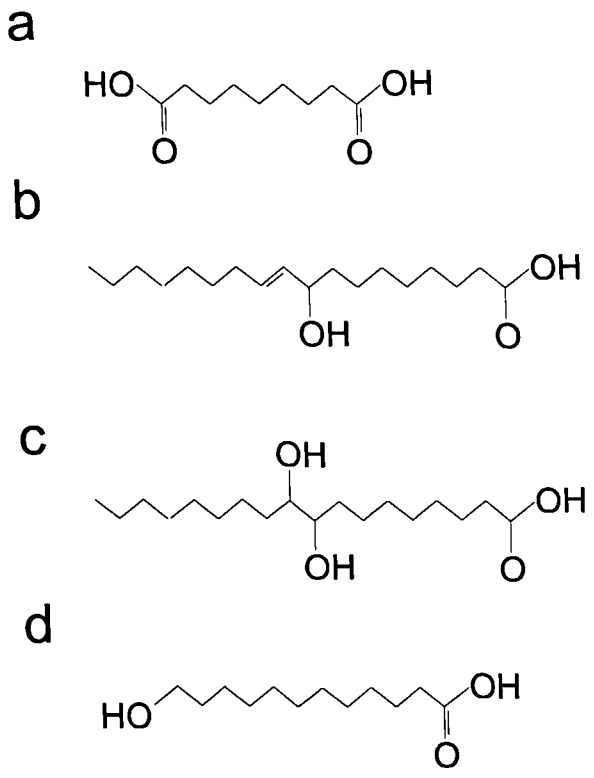


Figure 7.4 Structures of examples of fatty acid oxidation products. Structure identities are: (a) C_9 α,ω-dicarboxylic acid or azelaic acid; (b) 9-hydroxyoctadecenoic acid; (c) 9,10-dihydroxyoctadecanoic acid, and (d) ω-hydroxydodecanoic acid.

7.2 Previous work

The first decay experiments carried out in our laboratory were reported by Evershed *et al.* (1995a) and Charters (1996) who established that the incubation of potsherds in compost resulted in the decay of lipid absorbed into the ceramic matrix, despite the fact that the lipid components are absorbed within the porous fabric of the potsherds. Further experimental work illustrated the pattern of decay of lamb adipose fat under oxic and anoxic conditions over different time periods up to a maximum of 305 days. Several conclusions were drawn as a result of this work, including:

1. The rate of decay was dependant upon the experimental conditions imposed and decay proceeded at a greater rate in oxic conditions compared to anoxic conditions leading to a greater overall depletion of the lipid originally present and highlighting the greater potential for preservation of organic residues in anaerobic burial environments.

2. Different lipid species were also found to have different preservation potentials, with laboratory decay of lipid mixtures showing fatty acyl lipids are more prone to decay through hydrolysis and dissolution than long-chain alkyl components.

3. Despite the deleterious nature of the burial conditions, the distributions of acyl lipid components observed during decay reflected the range and distributions of components present in archaeological fat extracts.

4. $\delta^{13}C$ values obtained from the acid and neutral fractions of potsherd extracts prior to and following a 100 day incubation period (oxic and anoxic) indicated that no significant alterations in isotopic composition had occurred (e.g. due to bacterial input). On this basis it was concluded that the $\delta^{13}C$ values of individual fatty acids provide a robust criteria for use in distinguishing between different commodities processed in archaeological vessels.

Presented in this chapter are the results of experiments designed to promote the decay of lipids absorbed in ceramic sherds under controlled laboratory conditions. Milk and olive oil were chosen due to the range of lipid components present and in view of the economic and

nutritional importance of these two commodities in antiquity. The results obtained complement previous work involving the decay of ruminant adipose fat (Evershed *et al.*, 1995a; Charters, 1996). In addition to the decay of these complex natural mixtures, experiments were designed to monitor the decay of pure triacylglycerols, namely tristearin and triolein absorbed in potsherds. This provided a convenient means of assessing possible contributions from micro-organisms active in the degradation of the absorbed lipid. The experimental set-up used was based on previous work by Charters (1996).

The laboratory decay of lamb fat, initially set up by Charters in our laboratory, has been continued as part of this study with the analysis of sherds which have been subject to long-term incubation. The aims were to determine: (i) over what time period lipid is retained in the ceramic sherd, (ii) how the distributions of components are affected by long-term burial, and (iii) whether the $\delta^{13}C$ values of individual lipid components remain unaltered. The results of selected experimental work carried out by Charters (1996) are discussed in detail in the following sections of this chapter in relation to new data obtained.

7.3 Laboratory decay of lamb adipose fat

In order to determine the robustness and thus the reliability of stable carbon isotope ratios during the decay of fats and also to observe changes in the relative distributions of lipid components present, analyses were carried out on sherds from the same experiment as described by Charters (SIM 7, 305 day experiment; 1996) after an incubation interval approaching 3 years.

7.3.1 Dosing of sherds and experimental design

The potsherds used in these experiments come from replica pottery vessels, wheel-thrown and made from a mixture of pot clay (1137; Keuper Marl, Staffordshire) and sand (3:1, v/v) to provide a porous fabric. Lamb adipose fat (ca. 300 g) was boiled in water up to the shoulder of a replica vessel for 2 hr 30 mins (repeated 5 times) and the vessel subsequently broken up into pieces prior to incubation under anoxic and oxic conditions. In addition, experiments were set up where dosed sherds were autoclaved and incubated under oxic and anoxic conditions. A description of the experimental set up is given in Charters (1996).

Sherds were sampled following 10, 25, 50, 100 days by Charters (1996) and after 1300 days as part of this study. Sherds were cleaned and solvent extracted as described in Section 9.1.1.

7.3.2 Results of long-term decay experiments

7.3.2.1 Overall lipid distributions

The abundance of lipid ($\mu g\ g^{-1}$ of powdered sherd) remaining in the sherds after 1300 days of laboratory decay is shown in Table 7.1.

Table 7.1 Long-term laboratory decay of lamb adipose fat.

Sample	**Description of experiment**	**Total lipid content ($\mu g\ g^{-1}$)**
SIM 7 305 day expt.[1]		
ANLA1	Anoxic lamb (rep 1)	1282
ANLA2	Anoxic lamb (rep 2)	802
ANAUTLA1	Anoxic autoclaved lamb (rep 1)	3024
ANAUTLA2	Anoxic autoclaved lamb (rep 2)	1449
AERAUTLA1[2]	Oxic autoclaved lamb (rep 1)	2149
AERAUTLA2	Oxic autoclaved lamb (rep 2)	2673

[1] Reference in Charters (1996).

[2] Samples were analysed in order to determine the effect of autoclaving the dosed sherds prior to oxic decay.

In order to examine the change in total abundance of absorbed lipid during decay, the new data have been compared with data from the 40 day experiment (SIM 6) described by Charters (1996). Experimental conditions and set-up were exactly the same as in the 305 day experiment (but no quantitative data was available from the 305 day experiment). Figure 7.5 shows the decline in total lipid after 10, 25, 50, 100 and 1300 days of anoxic decay. The decline in lipid abundance is slower in sherds which were autoclaved prior to burial, even though they were incubated for the same duration [see **a** and **b**, Fig. 7.5]. The fluctuations seen in total lipid abundance at the start of the experiment can be attributed to the localised variation in porosity in the ceramic sherds and the effect of different surface areas of sherds on lipid absorbence.

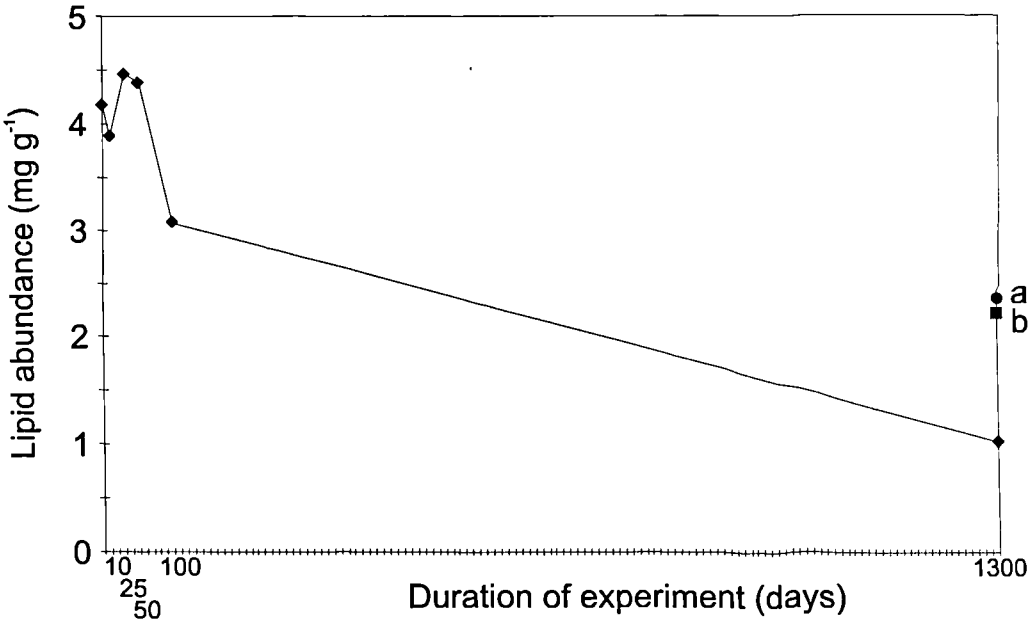


Figure 7.5 Change in the abundance (mg g^{-1}) of total lipid in the laboratory decay of lamb adipose fat absorbed in sherds from replica vessels incubated under anoxic conditions. The stages up to and including day 100 have previously been reported by Charters (1996). The data obtained for the long term decay of lamb adipose which had been autoclaved (along with the burial compost and decay vesicle) prior to the experiment have also been shown: (a) refers to oxic decay, and (b) anoxic decay.

The anoxic decay experiments showed a significant proportion of lipid remained even after ca. 3 years of incubation. The effect of oxic decay over this time period was not determined, however experimental work by Charters (1996) has shown the rate of decay to be significantly greater under oxic conditions. Autoclaved samples yielded a higher quantity of lipid after 1300 days of incubation, perhaps reflecting the effect of denaturing the enzymes present in the lamb fat which naturally enhance decay, or due to the elimination of bacterial populations in the soil matrix both of which may result in a reduction in the rate of decay.

After 1300 days of anoxic decay, a substantial quantity of absorbed lipid remained, whereas in the oxic decay experiment the majority of lipid had been degraded after 200 days. After 1300 days the overall abundance of absorbed lipid present at T0 in the lamb adipose decay experiment had decreased by approximately 75% under anoxic conditions. The abundance of lipid remaining in the autoclaved sample incubated under the same conditions and for the same duration was found to be 29% higher than in the sample which had not been autoclaved.

Figure 7.6 shows the changes in relative abundances of free fatty acids and triacylglycerols over time. As noted by Charters (1996), the intact triacylglycerols predominate initially, however, as decay progresses the decline in triacylglycerol abundance is mirrored by an increase in the abundance of free fatty acids. After 1300 days of incubation the ratio of free fatty acids to intact triacylglycerols is significantly greater. The pattern of decay seen in both the oxic and anoxic experiments is similar, however, the rate of anoxic decay is more than 6 times slower, with a substantial abundance (>800 μg g^{-1}) of lipid remaining after 1300 days.

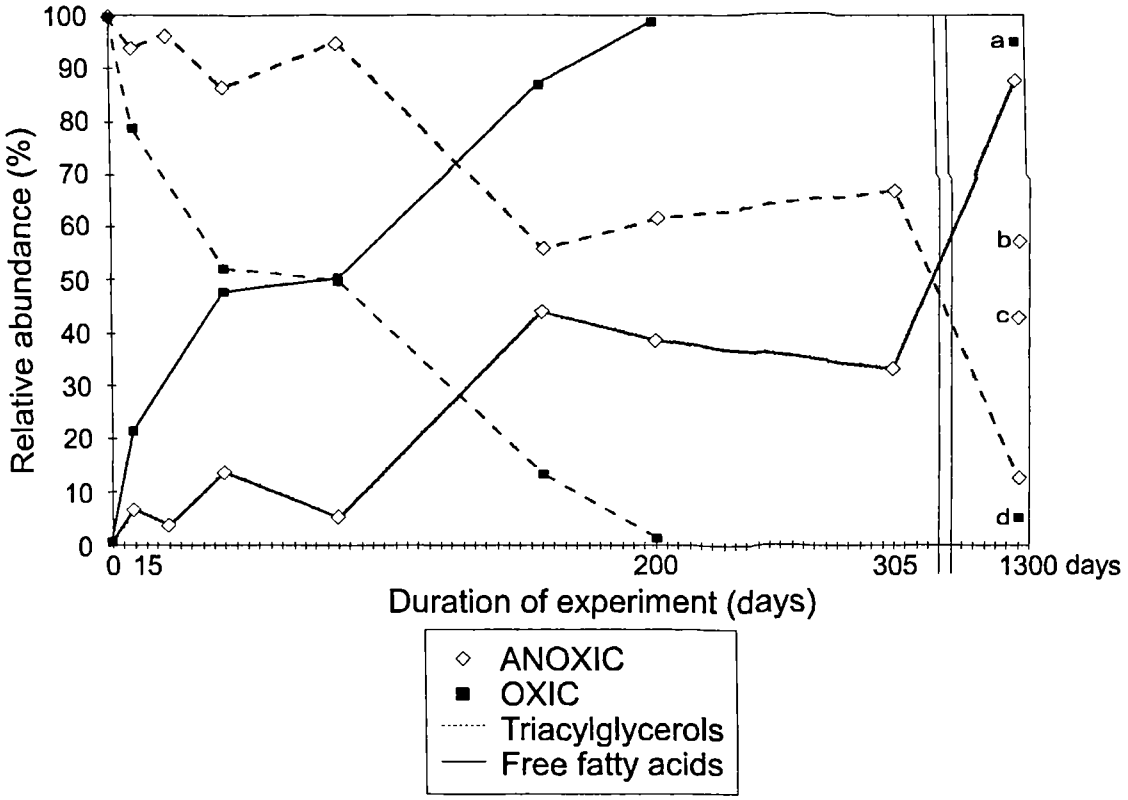


Figure 7.6 Change in the relative abundances (%) of total triacylglycerols and total free fatty acids in the lipid extracts from the experimental sherds over time. The stages up to and including day 305 have previously been reported by Charters (1996). The quantitative data obtained for duplicate samples of lamb adipose fat under anoxic conditions (open diamonds) and lamb adipose fat under oxic conditions (filled squares) are shown. The data obtained for lipid abundances in autoclaved samples (along with the burial compost and decay vesicle) after 1300 days have also been shown: (a) and (d) refer to abundances of free fatty acids and triacylglycerols, respectively, and (b) and (c) refer to abundances of triacylglycerols and free fatty acids, respectively.

7.3.2.2 Free fatty acid distributions

The distributions of the predominant free fatty acids, namely $C_{14:0}$, $C_{16:0}$, $C_{18:0}$ and $C_{18:1}$, varied according to the incubation conditions, with the relative abundance of the mono-unsaturated C_{18} significantly reduced in the anoxic and autoclaved oxic experiments after 1300 days (Fig. 7.7). In the autoclaved anoxic experiment the distribution remained essentially the same as at Day 10. It would appear that although the rate of decay is lower in the anoxic than the oxic experiments, the rate is slower still in the autoclaved anoxic experiment, with hydrolysis still resulting in the slow release of the $C_{18:1}$ component long after this has ceased in the other experiments. The oxic conditions apparently encourage oxidation of the mono-unsaturated C_{18} component which is not seen in the anoxic conditions.

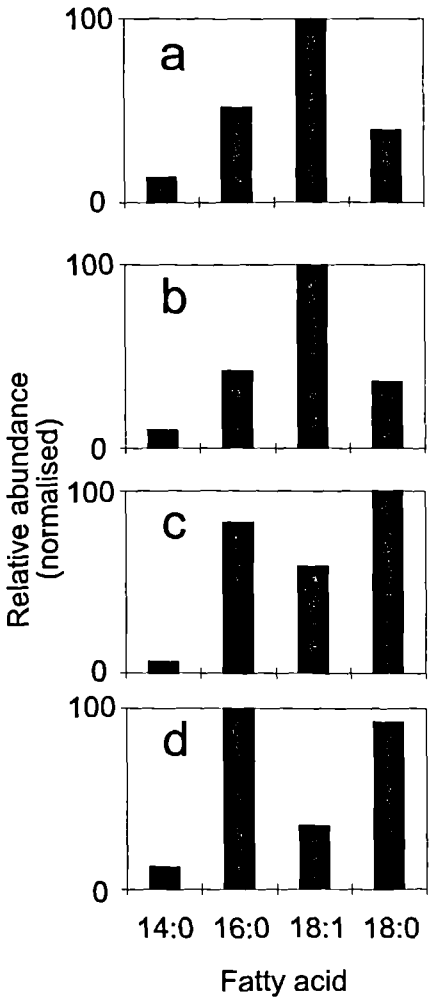


Figure 7.7 Free fatty acid distributions at Day 10 (a) and after 1300 days of incubation under autoclaved anoxic (b); anoxic (c), and autoclaved oxic (d) conditions.

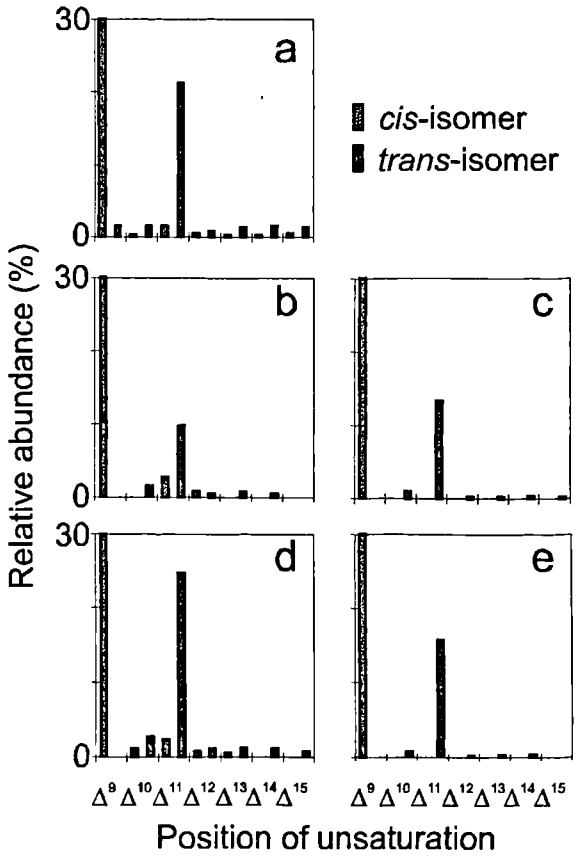


Figure 7.8 Distributions of positional isomers of the $C_{18:1}$ fatty acid in lamb fat (a) from fresh reference material; (b) absorbed into the body sherd of a replica vessel by successive boilings (see text for further details); (c) absorbed into the base sherd of a replica vessel; (d) absorbed into the body sherd of a replica vessel, autoclaved and incubated for 1300 days under oxic conditions, and (e) absorbed into the body sherd of a replica vessel and incubated for 1300 days under anoxic conditions.

The mono-unsaturated C_{18} fatty acids in the solvent extracts of the long-term laboratory decay experiments were derivatised to FAMEs, prepared as DMDS aducts and analysed by GC/MS to determine the distributions of positional and geometric isomers present. Figure 7.8 shows the variation in abundance of $C_{18:1}$ positional isomers between fresh unprocessed lamb, lamb subjected to excessive heat during boiling over an open flame, and lamb following laboratory decay. Sample (b) is from the body sherd of a replica vessel used to boil lamb on successive occasions; this sherd has been stored in the freezer (<-20 °C) for the length of the decay experiments. Sample (c) is from the base of the same vessel and thus was subject to intense heating during the processing. Samples (d) and (e) are body sherds from the same replica vessel, however, the former was autoclaved following impregnation with lipid and then incubated for 1300 days under oxic conditions, and the

latter had been incubated for 1300 days under anoxic conditions. No oxic or autoclaved anoxic samples were available for analysis. Figure 7.8 (a) shows the distribution of isomers in fresh lamb fat for comparison.

Lamb fat absorbed into the body sherd of the replica vessel (EV6; Charters, 1996) and subsequently stored in the freezer was still found to contain the range of *trans*-isomers and the *cis*-Δ^{11} and *cis*-Δ^{12} isomers present in the fresh fat, however, none of the other *cis*-configured isomers were present. There appeared to have been some loss during storage and almost certainly during the original processing of the meat in the vessel. This was certainly the case in the base sherd (c) where none of the *cis*-configured isomers were present. The effect of direct heat at the base of the vessel appeared to have significantly altered the original distribution, and shows the relatively high susceptibility of *cis*-configured fatty acids to thermal decomposition.

Although the range of $C_{18:1}$ components in the autoclaved oxic decay experiment had been altered by the loss of the *cis*-Δ^{14} and Δ^{15} isomers, the distribution was still recognisable as deriving from a ruminant fat. Anoxic decay lead to the more advanced decay of the *cis*-isomers, resulting in a profile similar to that seen in the sherds subject to thermal degradation. The preservation afforded to the autoclaved oxic decay samples indicates either that bacteria normally present in the residue contribute to the decay of *cis*-isomers or that enzymes active in the fats are denatured by autoclaving which reduced the rate at which decay occurs.

The decay experiments confirm that in degraded ruminant lamb fat the predominant isomers will be the *trans*-Δ^{11} and *cis*-Δ^{9}, with their relative abundance dependant on the extent of decay of the more labile *cis* isomer. The ratio of *trans* acids were found to be similar before and after laboratory decay due to their relatively high stability and provide a distribution which can be used to characterise a degraded ruminant fat.

7.3.2.3 Triacylglycerol distributions

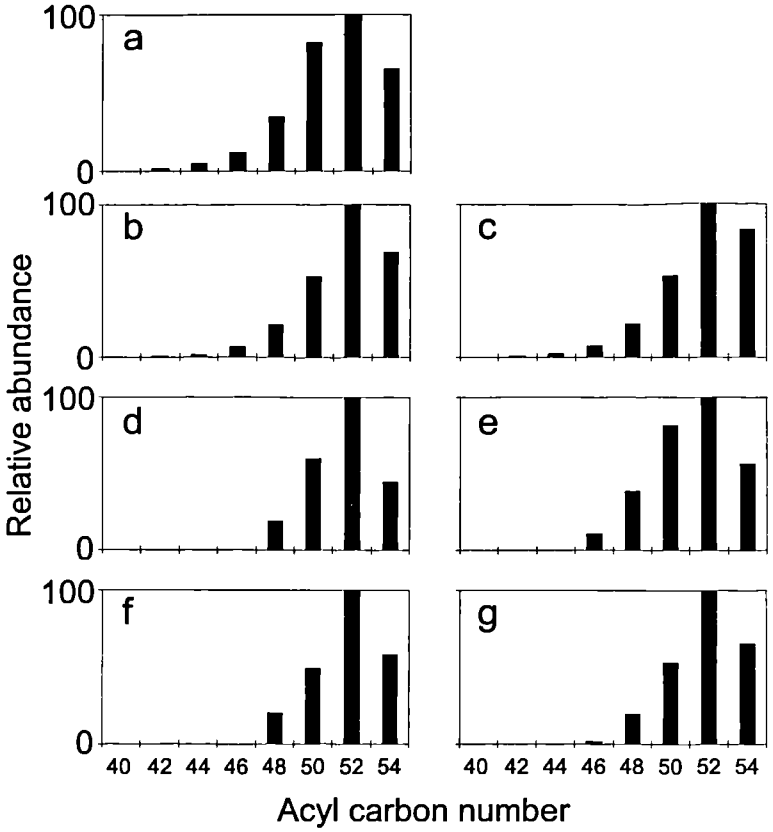


Figure 7.9 Distributions of intact triacylglycerols in total lipid extracts of lamb fat at T0 (a) compared with distributions resulting from 1300 days of laboratory decay under autoclaved anoxic conditions, rep. 1 (b) and 2 (c); autoclaved oxic conditions, rep. 1 (d) and 2 (e), and anoxic conditions, rep. 1 (f) and 2 (g).

Figure 7.9 shows the distributions of intact triacylglycerols in lamb adipose fat extracts at T0 and after long-term decay (ca. 3 years). No significant difference is apparent in the relative abundances of the triacylglycerol components, however in the majority of experimental conditions the C_{50} and lower carbon number components appear to have decreased relative to the C_{52} and C_{54} components. Interestingly, the lower carbon number triacylglycerols (C_{42}, C_{44} and C_{46}) are present in both the replicate samples from the autoclaved anaerobic decay experiments in approximately the same abundance as in the lamb fat at T0, whereas in all the other experiments (including the autoclaved oxic experiment) these lower carbon number components have been lost. This reflects the generally slower rate of decay which has been observed in the autoclaved anoxic experiments, indicating that the presence of oxygen and the effect of autoclaving the samples prior to decay combine to reduce the overall rate of decay.

7.3.2.4 Stable carbon isotope ratios of *n*-alkanoic acids

The stable carbon isotope values of the $C_{16:0}$ and $C_{18:0}$ fatty acids in the lamb fat were measured before and after incubation to determine whether the values were subject to change whilst the overall depletion of the lipid was occurring. The results are shown in Table 7.2. In fact, no change in the $\delta^{13}C$ values of the individual components was seen after 1300 days of decay under aerobic or anaerobic conditions. The data confirm that the contribution from bacterial lipids is extremely minor, since if bacteria were reworking fatty acids (and no other carbon source was available) then one would expect the bacterial fatty acids to be more depleted in ^{13}C. The results from these long-term experiments are very encouraging, justifying the use of stable carbon isotope ratios as a robust parameter for use in the assignment of animal fat origin.

Table 7.2 $\delta^{13}C$ measurements of *n*-alkanoic acids in lamb fat at T0 and following 1300 days of laboratory decay under the conditions given.

Sherd code	**Description**	**Time incubated**	***n*-alkanoic acid (‰)**	
			$C_{16:0}$	$C_{18:0}$
EV6 SB3 1	Body sherd from replica vessel used to cook lamb (rep. 1)	0 days	-30.1	-31.9
EV6 SB3 2	As above (rep. 2)	0 days	-30.1	-32.3
EV6 B BASE 1	Base sherd from replica vessel used to cook lamb	0 days	-29.8	-32.3
AN AUT 1	Body sherd as above, autoclaved and incubated in the laboratory under anoxic conditions (rep. 1)	1300 days	-30.0	-32.0
AN AUT 2	As above (rep. 2)	1300 days	-30.0	-32.2
AER AUT 1	Body sherd, autoclaved and incubated in the laboratory under oxic conditions (rep. 1)	1300 days	-30.1	-32.2
AER AUT 2	Body sherd, autoclaved and incubated in the laboratory under oxic conditions (rep. 2)	1300 days	-30.0	-32.3
AN 1	Body sherd incubated under anoxic conditions (rep. 1)	1300 days	-30.0	-32.5
AN 2	As above (rep. 2)	1300 days	-29.8	-32.3

7.4 Laboratory decay of mixtures of acyl lipids and epicuticular leaf wax components in potsherds

7.4.1 Experimental design

Experimental work initiated by Evershed and co-workers (1995) in order to observe changes in the relative abundances of mixtures of lipid components absorbed into the fabric of ceramic sherds during decay has been continued to observe the processes of decay over a longer term. The laboratory-based experiments were set up under oxic and anoxic conditions and initially sampled over a 125 day incubation period. A 25 mg ml^{-1} solution of lamb fat and cabbage leaf wax mixture (2:1 v/v) was prepared and the sherds dosed in the fat/wax mixture and ultrasonicated to facilitate absorption. Samples of the sherds dosed in the fat/wax mixture were taken at T0 and after days 10, 25, 50 and 125 days and analysed for lipid residues as described in Section 9.1.1. As part of this study, sherds were also sampled after 1120 days of incubation.

7.4.2 Results of fat and wax decay experiments

7.4.2.1 Overall lipid distributions

The results of lipid analysis of sherds following 1120 days of laboratory decay under oxic conditions are shown in Table 7.3.

Table 7.3 Long-term laboratory decay of lamb fat/leaf wax mixture.

Sample	Description	Total lipid content ($\mu g\ g^{-1}$)
SIM 8 125 day expt.[1]		
ANF&W1	Fat and wax mixture; anoxic (rep 1)	454
ANF&W2	Fat and wax mixture; anoxic (rep 2)	241

[1] Reference in Charters (1996).

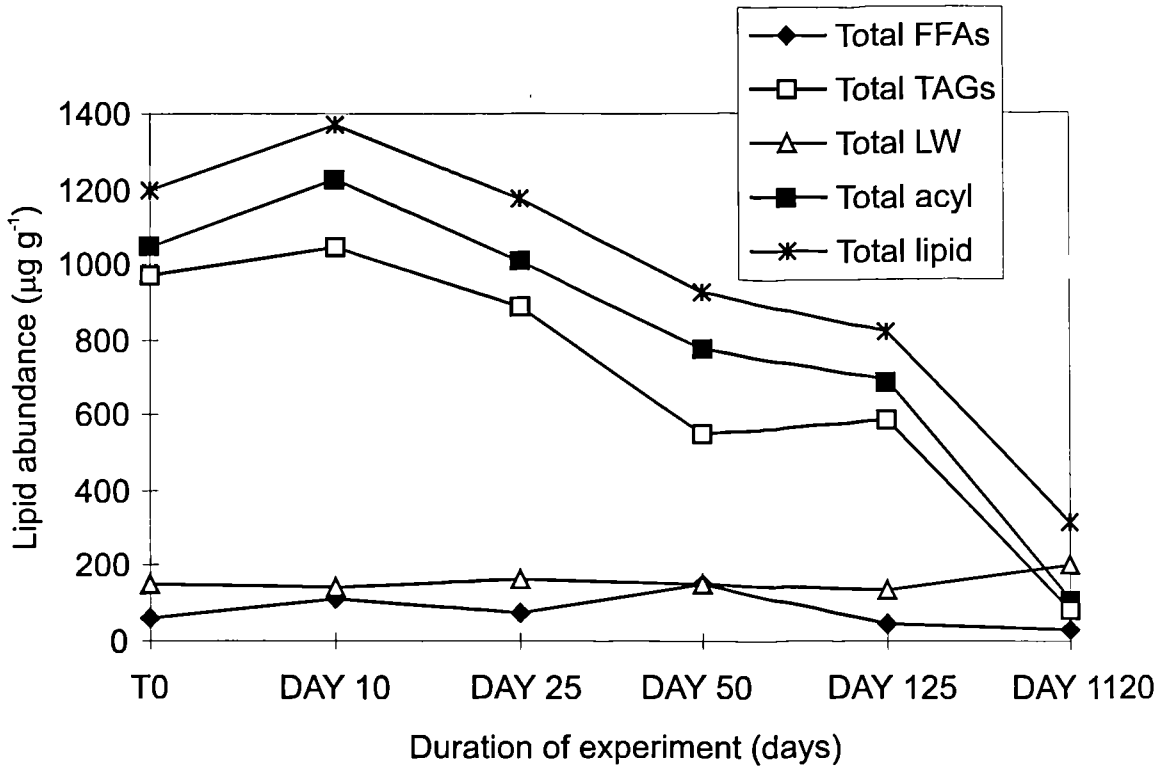


Figure 7.10 Changes in the abundances of acyl lipid, leaf wax components (alkanes, alcohols and ketones) and total lipid present in the fat/wax experimental sherds over time. The stages up to and including day 125 have previously been reported by Charters (1996).

The total abundance of the leaf wax components, including alkanes, alcohols and ketones, remains remarkably consistent throughout the course of the experiment (Fig. 7.10) whereas a significant change is seen in the abundance of intact triacylglycerols present in the lamb fat. Figure 7.11 shows that the distribution of triacylglycerols remains relatively consistent throughout the experiment, even though their overall abundance is greatly reduced. The free fatty acids remain in relatively low abundance throughout the course of the fat and wax decay experiment and are further reduced by the later stage of the experiment (Fig. 7.8).

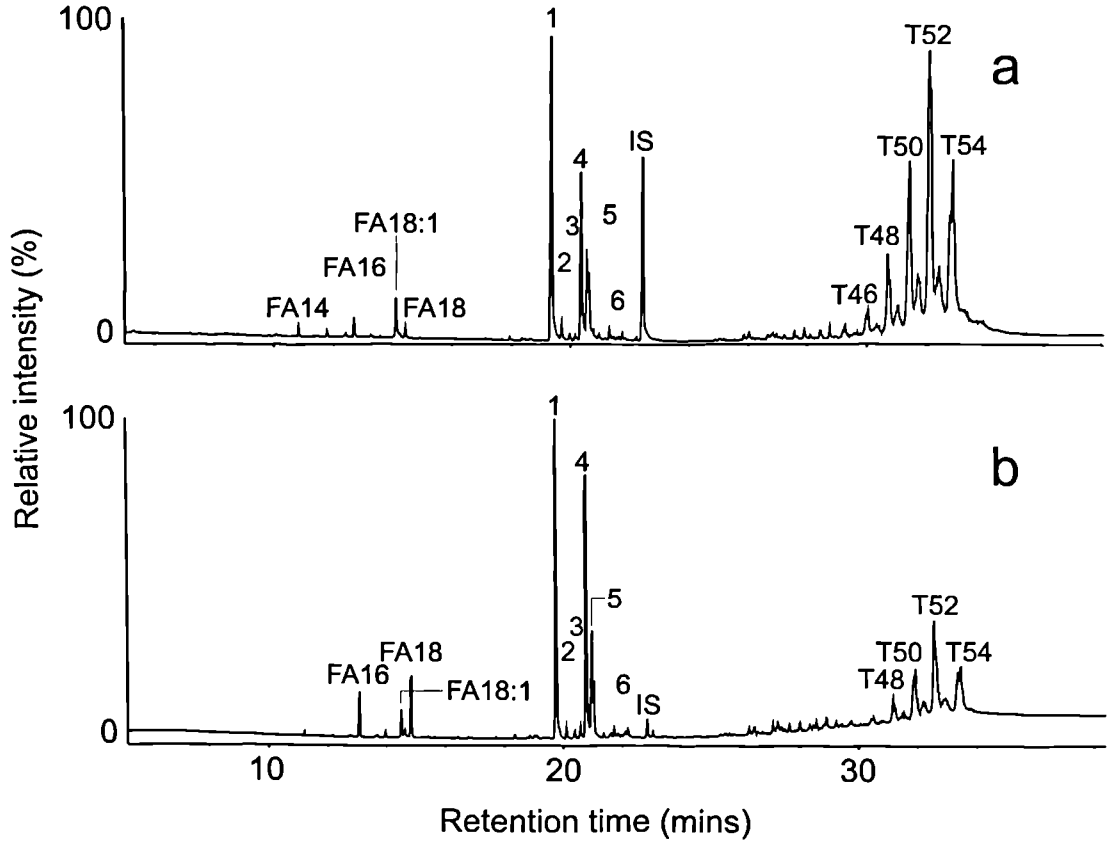


Figure 7.11 Changes in the abundances of lipid components present in the fat/wax experimental sherds (a) at T0 and (b) after 1120 days of anoxic decay in the laboratory. Peak identities are: FA16 and FA18, free fatty acids with 16 and 18 carbon atoms, respectively; FA18:1, free fatty acid with 18 carbon atoms and one double bond; **1**, nonacosane (C_{29}); **2**, long-chain primary alcohol (C_{26}); **3**, long-chain primary alcohol (C_{27}); **4**, nonacosan-15-one (C_{29}); **5**, nonacosan-15-ol (C_{29}); **6**, hentriacontane (C_{31}); **7**, long-chain primary alcohol (C_{28}); IS, internal standard (*n*-tetratriacontane), and T48 to T54, triacylglycerols containing 48 to 54 acyl carbon atoms, respectively.

Figure 7.11 shows a comparison of the leaf wax and lamb fat mixtures at T0 and after 1120 days. In the latter the leaf wax components are significantly better preserved in comparison with the lamb fat triacylglycerols. The free fatty acids remain in low abundance throughout the experiment. Despite the continued hydrolysis of triacylglycerols, the free acids are decaying faster than they can accumulate.

7.4.2.2 Distributions of leaf wax components

Figure 7.12 shows the relative abundances of leaf wax components preserved in replicate sherds following 1120 days of anoxic decay. The distribution of components is remarkably consistent between replicates even after three years of incubation. It is very encouraging to see that residues absorbed in pottery vessels decay in a systematic fashion which results in a characteristic distribution of lipid components. This result further validates the interpretations which have been made based on relative abundances of components.

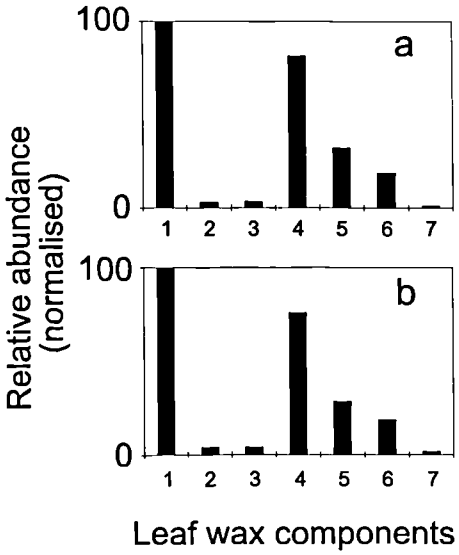


Figure 7.12 Relative abundances of leaf wax components in replicate samples (a and b) from the 1120 day anoxic decay experiment. The peak identities are: **1**, nonacosane (C_{29}); **2**, long-chain primary alcohol (C_{26}); **3**, long-chain primary alcohol (C_{27}); **4**, nonacosan-15-one (C_{29}); **5**, nonacosan-15-ol (C_{29}); **6**, hentriacontane (C_{31}), and **7**, long-chain primary alcohol (C_{28}).

Figure 7.13 shows the effect of anoxic decay on the relative abundances of the 3 major and diagnostic leaf wax components present in *Brassica oleracea* (cabbage). The nonacosan-15-ol has been reduced in abundance after 1120 days of incubation compared to the start of the experiment in relation to the abundances of the other components. The greater chemical reactivity of the alcohol functionality probably accounts for its preferential decay compared to the relatively resistant *n*-alkane and ketone components. The new data confirm observations first made by Charters (1996) and the comparable results obtained from replicate analyses indicate that under controlled conditions the decay process follows a reproducible path.

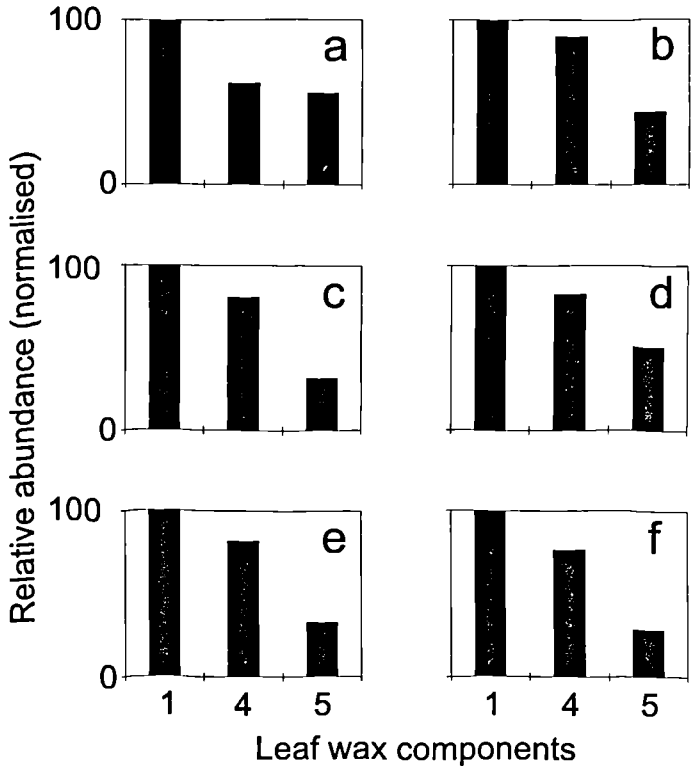


Figure 7.13 Relative abundances of leaf wax components in mixtures of fat and wax absorbed in replica ceramic sherds following laboratory decay after (a) 10 days; (b) 25 days; (c) 50 days; (d) 125; (e) 1120 days (rep. 1), and (f) 1120 days (rep. 2). The peak identities are: **1**, nonacosane (C_{29}); **4**, nonacosan-15-one (C_{29}), and **5**, nonacosan-15-ol (C_{29}).

7.4.2.3 Distributions of free fatty acids

Figure 7.14 shows the relative abundances of the free fatty acids present in the extracts from the fat/wax mixtures at different stages of the decay experiment. In the earlier stages of decay there was a high abundance of the free $C_{16:0}$ and $C_{18:1}$ components, whereas after 25 days the abundance of $C_{16:0}$ was reduced relative to the other components. After 1120 days the proportion of $C_{18:1}$ was greatly reduced and the remaining intact acyl lipid comprised a greater proportion of $C_{18:0}$ relative to the other fatty acids. In the later stages of the experiment it appears that hydrolysis yielded a higher proportion of the $C_{18:0}$ component. Significantly, the different rates of decay of the fatty acids have resulted in the alteration of the $C_{16:0}/C_{18:0}$ fatty acid ratio. The ratio was greater than unity at the start of the experiment but had changed to 0.7 after 1120 days of anoxic decay (Fig. 7.15). This reversal in the $C_{16:0}/C_{18:0}$ ratio clearly needs to be taken into account when making interpretations based upon distributions of lipid components in remnant fats.

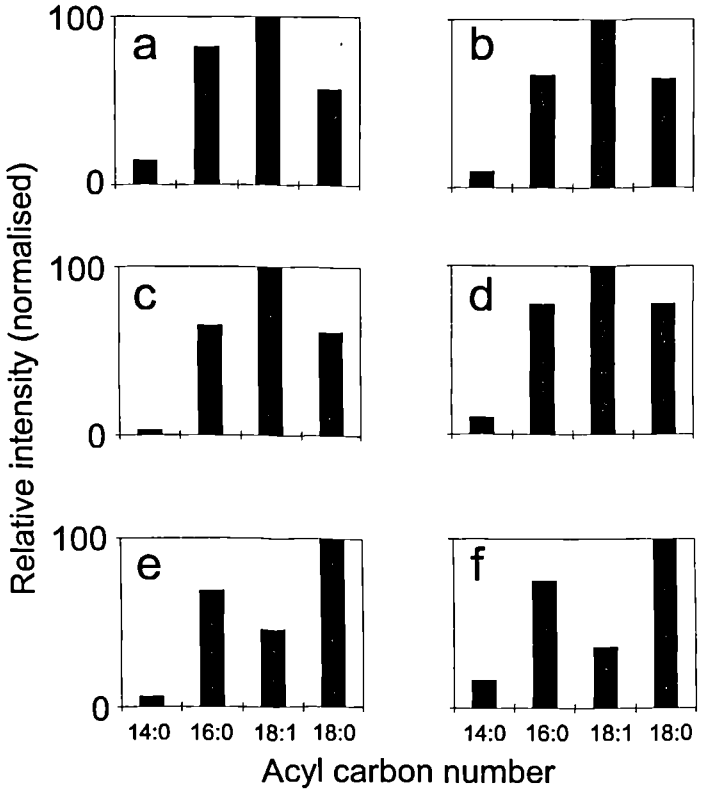


Figure 7.14 Changes in the abundances of free fatty acid components present in the fat/wax experimental sherds after (a) 10 days; (b) 25 days; (c) 50 days; (d) 125; (e) 1120 days (rep. 1), and (f) 1120 days (rep. 2). The stages up to and including day 125 have previously been reported by Charters (1996).

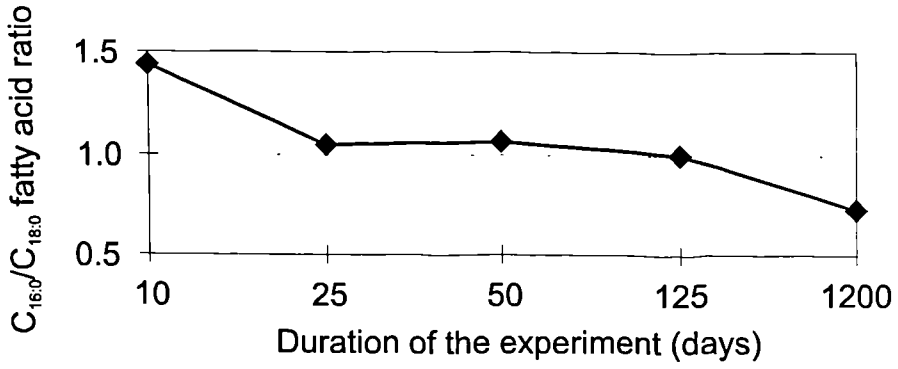


Figure 7.15 The $C_{16:0}/C_{18:0}$ free fatty acid ratio in fat and wax mixtures at progressive stages of anoxic decay.

7.5 Laboratory decay of milk fat and olive oil

7.5.1 Dosing of sherds and experimental design

Sherds (approx. 2 g) were prepared by dosing in solutions of milk (white goat), olive oil [pure virgin; 25 mg ml^{-1} solution in dichloromethane (DCM)]. The absorption of lipid was facilitated by sonication for 2 x 20 min. One sherd from each was kept for analysis at T0. The sherds were dried to constant weight at room temperature before burial in flasks (Duran; 250 ml) of "mushroom compost" (Magnolia Brand; mushroom humix manure). The flasks were plugged with extracted cotton wool in order to allow diffusion of air. Sherds dosed with lipid were incubated in clean, compost-free flasks as controls. The potsherds were then incubated at 30°C and removed at intervals for the extraction of organic residues (Section 9.1.1). Details of the fat and oil decay experiments are given in Table 7.4.

Table 7.4 Summary of laboratory decay experiments carried out under passive oxic conditions to investigate the degradation of lipids absorbed in archaeological potsherds.

Substrate	Internal standard[1] (μg)	Incubation temperature (° C)	Sampling intervals (days)
Olive oil	80	30	0, 10, 20, 67, 95
Milk	80[2]	30	0, 5, 10, 15, 25
Controls[3]			
Olive oil	80	30	15
Milk	30	30	15

[1] Added per 2 g of powdered sherd
[2] 20 μg of internal standard added at day 25
[3] Sherds incubated in a clean flask without compost

7.5.2 Results of the laboratory decay of reference fats and oils

7.5.2.1 Milk fat

In its undegraded form (composition at T0) milk fat consists predominantly of triacylglycerols ranging from C_{26} to C_{54} (number of acyl carbon atoms) containing fatty acids between C_4 and C_{20} (Breckenridge and Kuksis, 1969; Smith *et al.*, 1968; Evershed, 1995). After only 10 days of laboratory decay of milk fat under oxic conditions a >95% reduction in the concentration of intact triacylglycerols present absorbed in the potsherds at T0 had occurred. The major decay process appeared to be hydrolysis of the triacylglycerols

resulting in the liberation of free fatty acids in the range C_8 to C_{20}, with the $C_{14:0}$, $C_{16:0}$ and $C_{18:0}$ components predominating (Fig. 7.16). Mono- and diacylglycerols were produced in relatively minor quantities. Hydrolysis of triacylglycerols is thought to have proceeded rapidly once the first fatty acid had been cleaved from the glycerol backbone since monoacylglycerols and diacylglycerols did not accumulate to any appreciable extent during the experiment. The quantitative data for this experiment are given in Table 7.5.

Table 7.5 Absolute concentrations of lipid components (μg g^{-1} of powdered sherd) present during laboratory decay of milk under oxic conditions.

Day	Free fatty acids					Triacylglycerols	Total lipid (μg g^{-1})
	$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{18:1}$	$C_{18:0}$		
0	0.0	0.0	2.4	2.2	0.0	314.9	391.5
5	0.7	4.6	19.2	19.2	8.2	19.6	71.5
10	0.0	0.3	4.3	4.2	2.0	5.1	15.8
15	0.0	0.2	1.5	1.1	0.6	5.8	9.2
25	0.0	0.2	0.6	0.7	0.4	5.5	7.3

As a result of dosing, 392 μg g^{-1} of lipid had been absorbed by the potsherd (T0). The total amount of lipid present decreased throughout the experiment with the total triacylglycerol concentration decreasing from 315 μg g^{-1} to only 20 μg g^{-1} between T0 and day 5 with a slower but gradual decrease thereafter. Figure 7.17 (a) shows a plot of the changing relative abundances of free fatty acids and intact triacylglycerols during the course of the laboratory decay. There was no significant change in the relative proportions of the major fatty acids ($C_{16:0}$ and $C_{18:0}$) during the course of the decay. Initially, the proportion of intact triacylglycerols decreased with a corresponding increase in the abundance of free acids; after 10 days there was a decrease in the rate of depletion of triacylglycerols relative to the free fatty acids [Fig. 7.17 (a)].

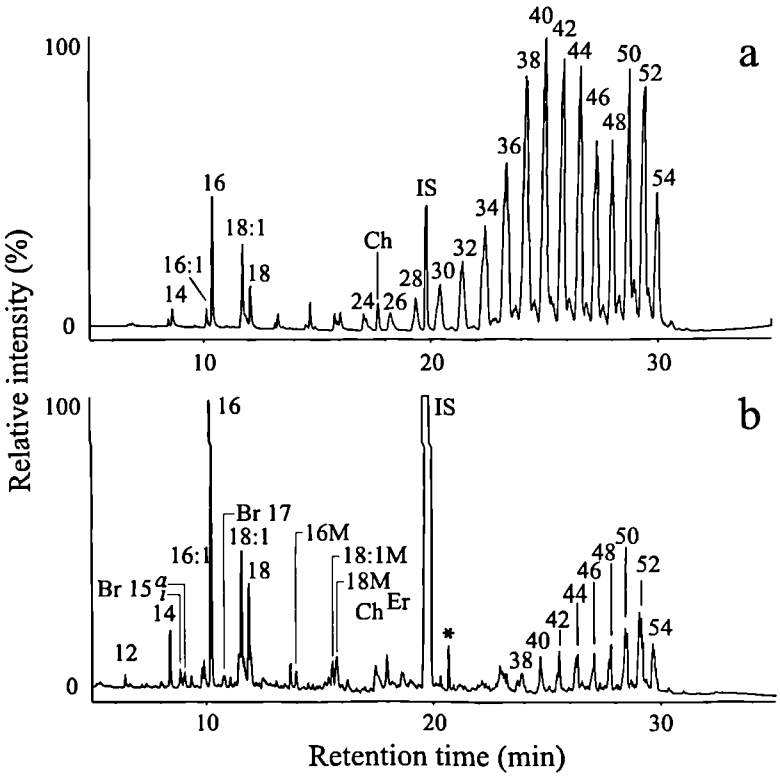


Figure 7.16 Partial HTGC profiles of the TLE of milk fat residues (a) at T0, and (b) after 25 days of laboratory decay. Peak identities are: 14, 16 and 18 correspond to *n*-alkanoic acids with 14, 16 and 18 carbon atoms, respectively; 16:1 and 18:1 correspond to monounsaturated fatty acids with 16 and 18 carbon atoms respectively; Br15 and Br17 are co-eluting *iso*- and *anteiso*-branched alkanoic acids containing 15 and 17 carbon atoms, respectively; 16M and 18M refer to monoacylglycerols consisting of acyl lipids with 16 and 18 acyl carbon atoms respectively; Ch and Er refer to cholesterol and ergosterol, respectively; IS refers to the internal standard, *n*-tetratriacontane; 26, 28, 30-54 etc. refer to triacylglycerols with 26, 28, 30-54 etc. acyl carbon atoms, respectively; * denotes plasticiser contamination.

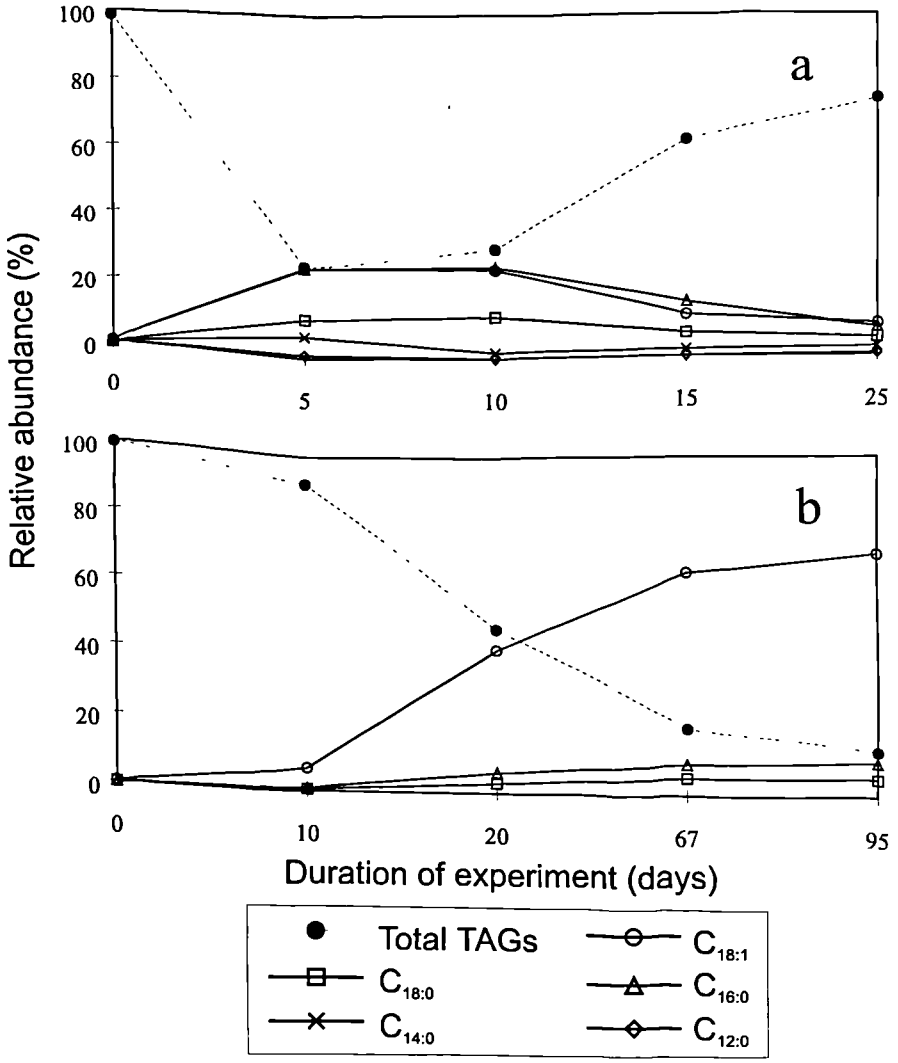


Figure 7.17 Relative abundances (%) of the lipid components of a) milk fat and b) olive oil during laboratory decay experiments.

The HTGC profiles of the lipid extracts show that during decay, alteration of the distribution of intact triacylglycerols occurred due to the early liberation of shorter-chain fatty acyl moieties (Fig. 7.18). By day 25 the lower molecular weight triacylglycerols (C_{26} to C_{44}) which originally comprised 66% of total triacylglycerols had been reduced to only 34%. Their longer-chain counterparts (C_{46} to C_{54}), which originally comprised 34% had increased to 67% of the total triacylglycerols [Fig. 7.18 (b)]. Furthermore, the shorter-chain free fatty acids characteristic of milk fats (C_4 to C_{12}) were largely undetectable and only a very small proportion of the original lipid remained, approximating to 7 $\mu g\ g^{-1}$. This evidence correlates with the data from the lamb decay experiments, where the lower carbon number triacylglycerols are hydrolysed in preference to those containing a higher proportion of longer-chain acyl moieties (see Fig. 7.9).

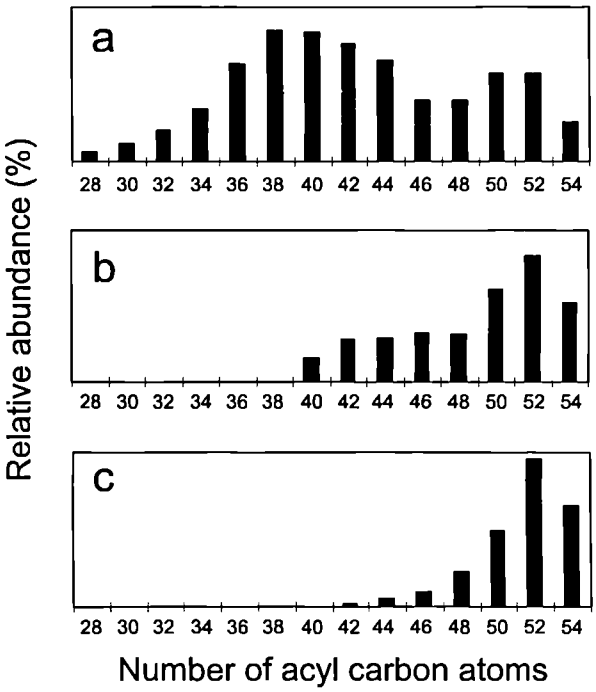
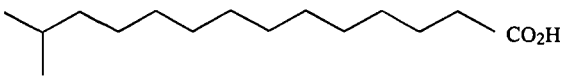
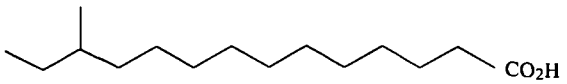


Figure 7.18 Triacylglycerol distributions in (a) fresh milk; (b) milk absorbed in an unglazed potsherd and degraded in the laboratory under oxic conditions for 90 days, and (c) fresh ruminant (ovine) adipose fat. The distributions were determined by HTGC of total lipid extracts (Evershed, 1995).

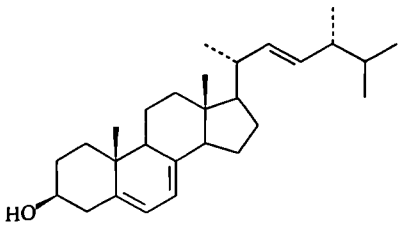
A significant abundance of branched-chain (*iso* and *anteiso*; Fig. 7.19 **I** and **II**) and odd-chain carbon number components was apparent in the degraded residue from day 5 onwards, however, their appearance was to be expected due to their natural occurrence in ruminant milk. In contrast, ergosterol [*(22E)*-ergosta-5,7,22-trien-3β-ol; Fig. 7.19 **III**] present in the degraded milk residues from day 5, does not occur naturally in milk and therefore must have derived from the yeast and fungi involved in the degradation process (Goad and Akihisa, 1997, and references therein).



I *iso*-$C_{15:0}$ fatty acid



II *anteiso*-$C_{15:0}$ fatty acid



III Ergosterol [(22*E*)-ergosta-5,7,22-trien-3β-ol]

Figure 7.19 Structures of branched-chain fatty acids (**I, II**; bacterial) and ergosterol (**III**; fungal).

7.5.2.2 Olive oil

Intact pure virgin olive oil (composition at T0) is composed predominantly of triacylglycerols bearing 50, 52 and 54 acyl carbon atoms, with $C_{18:1}$ the most abundant fatty acyl moiety. The major free fatty acids liberated during decay included the $C_{16:0}$, $C_{18:1}$ and $C_{18:0}$ components. The relative proportions of free fatty acids present did not alter significantly during the course of the experiment (Fig. 7.20 and Table 7.6). Initially >3700 $\mu g\ g^{-1}$ of lipid was present in the sherd (T0), 90% more than was absorbed by the potsherds in the milk experiment. The greater absorption of olive oil may have been facilitated by the solvent with which it was mixed, since in the milk experiment sherds were dosed in undiluted milk although it is most likely that the high fat content of the oil compared to the milk resulted in greater absorption within the pores and also adsorption to the exposed surfaces. A 60% decrease in the concentration of total extractable lipid was observed by day 15, with a >90% decrease of total lipid occurring by day 95 [Fig. 7.20 (b) and Table 7.6]. Close inspection of the lipid profiles revealed the appearance of straight- and branched-chain fatty acids not present in the oil at the start of the experiment (Fig. 7.21). The relative abundance of these compounds increased during the course of the experiment. However, even after 95 days of decay they were present in only very minor abundances, constituting <2% of the total free fatty acids present. The appearance of $C_{14:0}$, $C_{15:0}$ and $C_{20:0}$

components occurred together with smaller amounts of the branched-chain *iso*- and *anteiso*-$C_{15:0}$ and $C_{17:0}$ fatty acids known to be of bacterial origin. The components eluting between 12 and 14 mins in the gas chromatogram in Figure 7.21 have been identified by mass spectrometric analysis as hydroxyoctadecenoic acids, oxidation products of the mono-unsaturated C_{18} component abundant in the olive oil. **A** is a co-eluting mixture of two isomers, the 9-hydroxy, 10-ene and the 10-hydroxy, 8-ene, and **B** is a mixture of the 8-hydroxy, 9-ene and the 11-hydroxy, 9-ene. The structures of these components are shown in Figure 7.22 and their formation is been discussed in Section 7.7.

Table 7.6 Absolute concentrations of lipid components (μg g^{-1} of powdered sherd) present during laboratory decay of olive oil under oxic conditions.

Day	Free fatty acids			Acylglycerol components			Total lipid
	$C_{16:0}$	$C_{18:1}$	$C_{18:0}$	Mono-	Di-	Tri-	(μg g^{-1})
0	0	12.7	0	9.8	74.8	3696.7	3795.1
10	21.4	164.0	14.3	8.9	105.8	2214.6	2544.6
20	75.7	530.9	35.8	28.7	93.1	607.6	1405.7
67	31.3	223.9	17.4	4.2	9.4	67.4	366.9
95	24.6	175.3	12.6	1.4	3.8	32.6	262.0

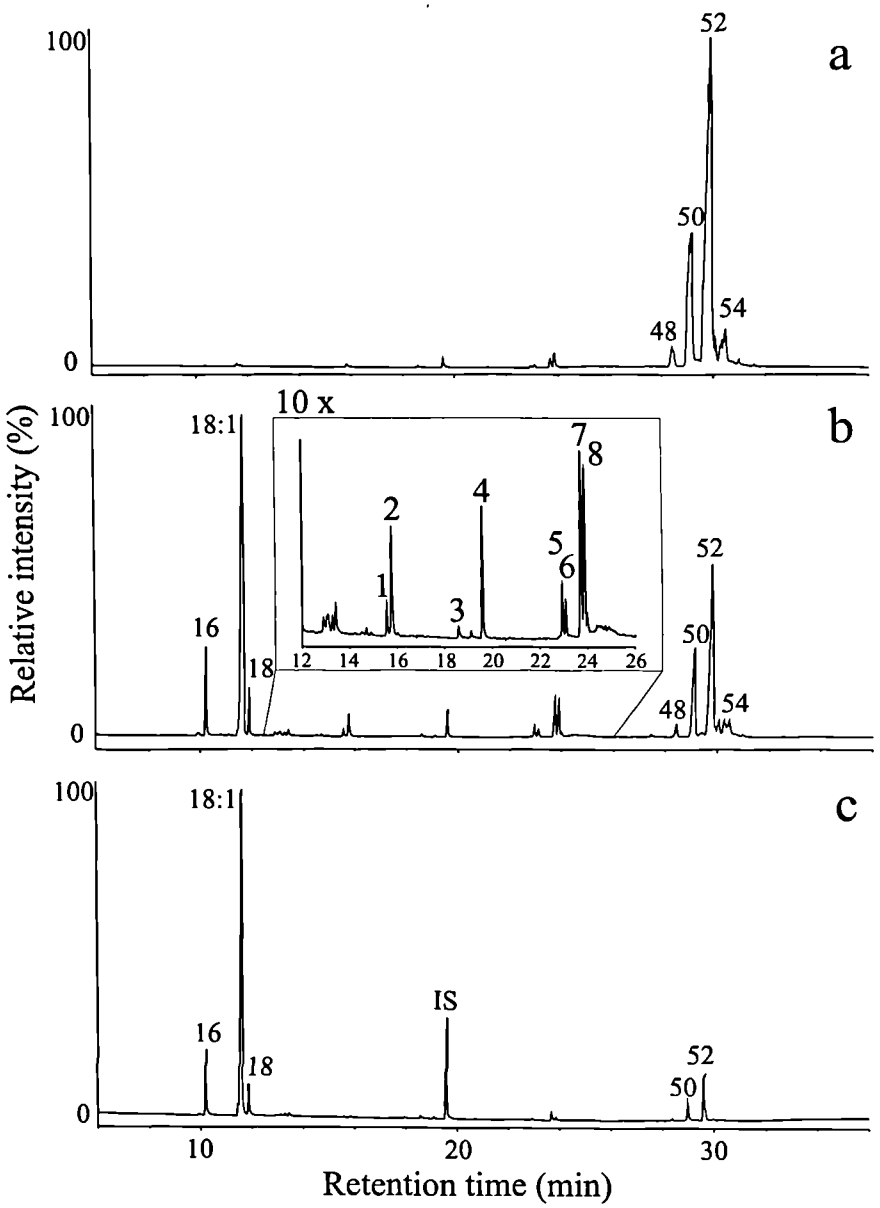


Figure 7.20 Partial HT-GC profiles of olive oil residues (a) at T0; (b) after 20 days, and (c) after 95 days of laboratory decay. Peak identities are the same as in Figure 7.16. Peaks in the expanded region of chromatogram (b) are: **1**, $C_{18:1}$ 1-monoacylglycerol; **2**, $C_{18:1}$ 2-monoacylglycerol; **3**, sitosterol; **4**, internal standard (*n*-tetratriacontane); **5**, $C_{16:0}$, $C_{18:1}$ diacylglycerol; **6**, $C_{16:0}$, $C_{18:0}$ diacylglycerol; **7**, $C_{18:1}$, $C_{18:1}$ diacylglycerol, and **8**, $C_{18:0}$, $C_{18:1}$ diacylglycerol.

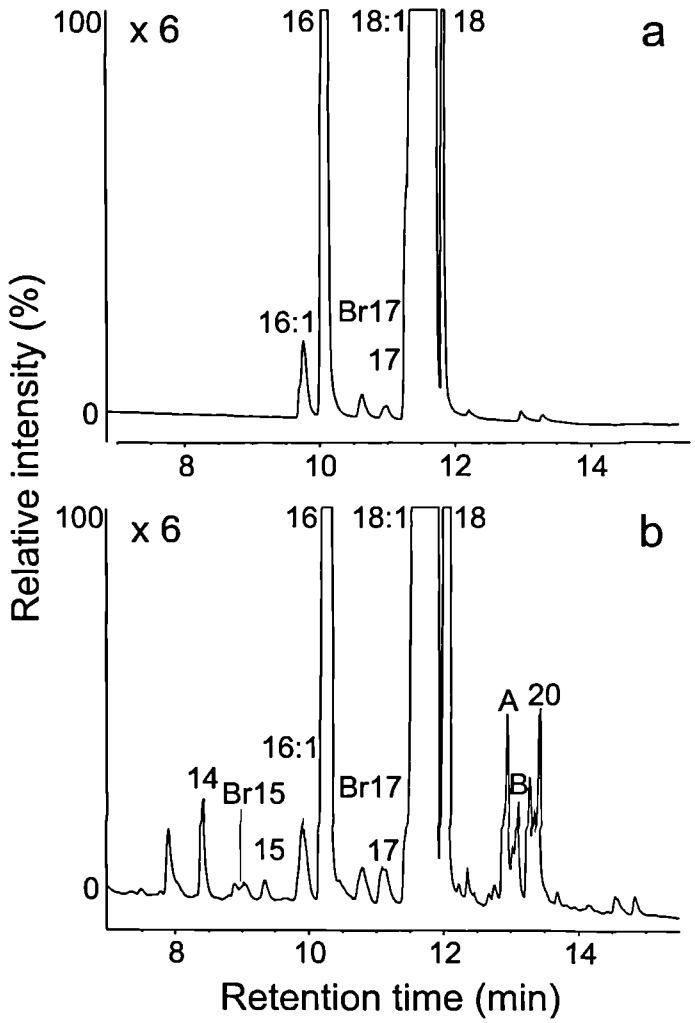


Figure 7.21 Partial HTGC profiles of free fatty acids from (a) saponified olive oil at T0, and (b) the acid fraction of olive oil after 95 days of laboratory decay. Peak identities are the same as in Figure 7.16. **A** and **B** refer to oxidation products, see text for further details. The scale has been expanded to show the detail of the minor peaks.

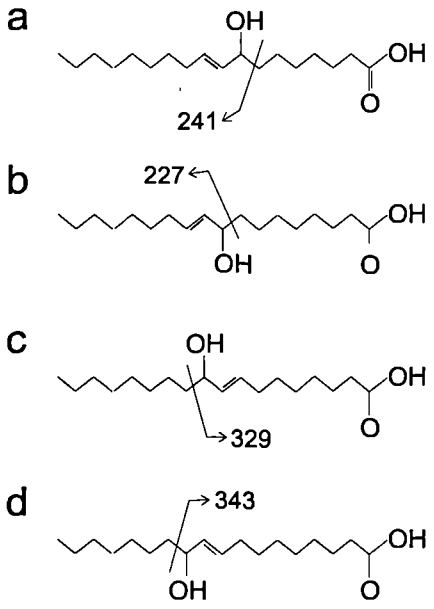


Figure 7.22 Structures and diagnostic MS fragmentations of hydroxyoctadecenoic acids identified in the acid fraction of olive oil after 95 days of laboratory decay: (a) 8-hydroxy-9-octadecenoic acid; (b) 9-hydroxy-10-octadecenoic acid; (c) 10-hydroxy-8-octadecenoic acid; (d) 11-hydroxy-9-octadecenoic acid.

7.6 Laboratory decay of pure triacylglycerols

7.6.1 Dosing of sherds and experimental design

Dosing of sherds was carried out as described previously (Section 7.5.1) using tristearin (10 mg ml^{-1} in DCM; Sigma T-5016) and triolein (10 mg ml^{-1} in DCM; Sigma T-7140). The experimental conditions are shown in Table 7.7.

Table 7.7 Experimental conditions for the laboratory decay of pure compounds.

Substrate	Internal standard[1] (μg)	Incubation temperature (°C)	Sampling intervals (days)
Tristearin	50	30	0, 6, 14, 32, 57, 84, 126
Triolein	50	30	0, 7, 14, 28, 57, 97, 126

7.6.2 Results of the laboratory decay of pure reference compounds

After 7 days of incubation under oxic conditions hydrolysis of triolein had resulted in the production of diacylglycerols, monoacylglycerols and free $C_{18:1}$. At first the rate of accumulation of mono- and diacylglycerols by hydrolysis of triacylglycerols appeared to

be more rapid than their rate of degradation (e.g. by further hydrolysis). This pattern of decay continued through to day 57 (Table 7.8). Between 57 and 97 days, the relative proportion of triolein remained constant (57.8% and 57.4%), while a decrease in the relative proportion of the diacylglycerols was observed. During this time the monoacylglycerols and free $C_{18:1}$ continued to increase in relative abundance.

Table 7.8 Compositional data from the laboratory decay of pure triolein (as percentage composition). The data given for mono-, di- and triacylglycerols represent the sum of each class of compound.

Duration of the experiment (days)	**Free $C_{18:1}$ (%)**	**Mono-acylglycerols (%)**	**Di-acylglycerols (%)**	**Tri-acylglycerols (%)**
0	nd[1]	nd	nd	99.2
7	2.4	0.3	6.0	90.6
14	6.7	nd	7.2	83.9
28	11.5	3.6	22.3	61.0
57	9.6	3.8	26.1	57.8
97	22.8	2.5	16.8	57.4
126	25.3	7.5	23.2	39.8

[1] nd=not detected

The same general trends occurred during the oxic decay of tristearin absorbed in sherds as for triolein, although the rate of degradation in the case of the former was slower. Diacylglycerols were produced after 6 days with monoacylglycerols and $C_{18:0}$ appearing between day 14 and day 32 (Table 7.9). After 57 days, additional minor components were detected in both experiments, as shown in the partial gas chromatograms displayed in Figure 7.23 (a) and (b). These components were identified as saturated, unsaturated and branched-chain fatty acids, including straight-chain $C_{14:0}$, $C_{15:0}$, $C_{16:0}$, $C_{17:0}$, $C_{18:0}$ and $C_{18:1}$, and branched-chain $C_{15:0}$ and $C_{17:0}$ (*iso-* and *anteiso-*); the minor components being dominated by the straight-chain $C_{14:0}$ and $C_{16:0}$ fatty acids.

Table 7.9 Compositional data from the laboratory decay of pure tristearin (as percentage composition). The data given for mono-, di- and triacylglycerols represent the sum of each class of compound.

Duration of the experiment (days)	**Free $C_{18:0}$ (%)**	**Mono-acylglycerols (%)**	**Di-acylglycerols (%)**	**Tri-acylglycerols (%)**
0	nd[1]	nd	0.3	98.9
6	nd	nd	2.2	95.8
14	nd	nd	9.0	90.0
32	6.2	0.6	6.5	86.7
57	6.5	1.6	14.1	77.0
84	17.0	6.2	34.0	41.0
126	32.0	6.8	31.7	24.5

[1] nd=not detected

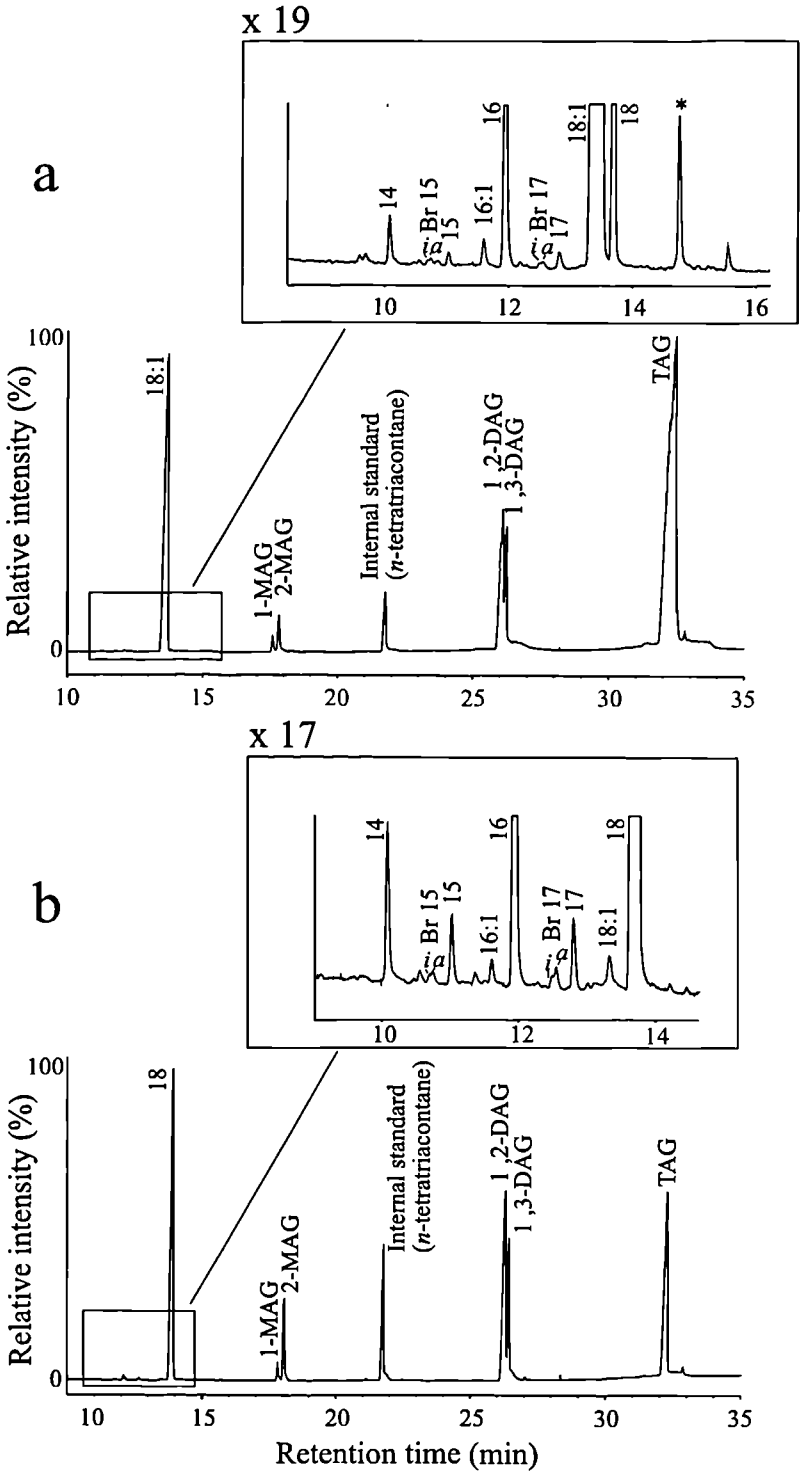


Figure 7.23 Partial HTGC profiles of (a) triolein after 97 days of laboratory decay and (b) tristearin after 126 days of laboratory decay. Regions of the chromatograms are expanded in order to show the distributions of minor components present. Peak identities are the same as in Figure 7.16, with the addition of 1-MAG and 2-MAG which refer to monoacylglycerols with the fatty acyl moieties esterified at the 1- and the 2-positions, respectively; 1,2-DAG and 1,3-DAG refer to diacylglycerols with fatty acyl groups esterified at the 1,2- and 1,3-positions, respectively, and * denotes plasticiser contamination.

7.7 Discussion

During the laboratory decay of pure triacylglycerols absorbed in sherds, both tristearin and triolein were seen to undergo partial hydrolysis to free fatty acids ($C_{18:0}$ and $C_{18:1}$, respectively), monoacylglycerols and diacylglycerols, possibly as a result of a combination of biological and chemical hydrolysis. The relative contribution of both processes involved is difficult to evaluate, although previous experiments (not involving potsherds) have shown a high rate of hydrolysis of tristearin during the first week of incubation due to the action of lipases (Hita *et al.*, 1996). This would also account for the slower rate of decay in the autoclaved lamb fat samples in which lipases would have been denatured prior to burial (Section 7.3.2.1). The absence of bacterial markers in the early stages of the experiments may suggest that chemical hydrolysis was responsible for initial degradation, however, the possibility cannot be ruled out that these markers are present in low abundance, but are swamped by the high concentrations of added lipid still present in the early stages of the experiment. Thus, bacterial markers might only be detectable after the major proportion of the utilisable lipid has been consumed and dead bacterial biomass is added to the organic matter in the sherds.

Once the cleavage of fatty acyl moieties from the glyceride backbone is initiated, the complete hydrolysis of acyl lipids and the subsequent decay of free fatty acids, e.g. *via* β-oxidation, is rapid. Therefore, the rate determining step in the decay of fats is apparently the cleavage (e.g. by chemical or enzymatic processes) of the first fatty acid moiety. Since micro-organisms are generally ubiquitous it is assumed that although they may be involved in the reworking and/or removal of free fatty acids, their involvement does not significantly influence the rate of decay, besides which, bacteria are thought to be physically larger than the majority of the pore spaces in a fired sherd. The role of enzymes within the fats and oils are thought to be significant in the initial depletion of lipid as shown by the slower depletion of autoclaved fats, and a major factor affecting triacylglycerol preservation would appear to be the effectiveness of entrapment within, or absorption to, the clay matrix. It may be that the protection provided by the clay matrix (either by physical entrapment or chemical bonding) is less effective in certain environmental conditions, e.g. where there are extremes of temperature, pH or wetting and drying. One can postulate that this is the reason why we have observed extremely variable preservation of lipid during the

analysis of residues in archaeological pottery from contemporary sites in different geographical locations.

The absence of oxygen cannot be the overriding factor which preserves lipids in archaeological pottery. This is reinforced by the fact that we also have recovered substantial amounts of lipid from unglazed ethnographic vessels which have not been buried. Thus lipid which is preserved over archaeological time scales must be preserved either by the physical protection provided by the clay matrix of the pot, as a result of becoming irreversibly bound to the clay surface during the original use of the vessel, or through polymerisation and the formation of macromolecules within the pot matrix. Although anoxic decay slows the initial loss of lipid it is not the main reason for the long term preservation of lipid. A combination of factors are likely to affect the physical entrapment of lipid within the pot wall, including the nature of the fabric of the pottery which will vary in friability, hardness, size or number of pore spaces and mineral content, etc.

In the laboratory decay of milk under oxic conditions, we have seen that the transformation of the lipid distribution in milk to one more closely resembling that of adipose fat occurs through preferential hydrolysis of the shorter-chain acyl moieties. This is due to reduced steric effects at the ester linkages of short-chain fatty acids in triacylglycerols compared with their long-chain counterparts (Balls *et al.*, 1937). Furthermore, once released from triacylglycerols, short-chain fatty acids are appreciably more water soluble (and volatile) than their long-chain counterparts (Gunstone *et al.*, 1986). Hence, the similarity of the composition of adipose fat and degraded milk fat indicates that caution is required in our interpretations of lipid distributions in archaeological fat residues, and previous assignments of animal adipose fats may need to be reconsidered since some of them may actually represent degraded dairy fats.

Since milk fat and other ruminant fats naturally contain such a complex mixture of lipid components, including branched-chain and odd-carbon number fatty acids, comparison of the decay products of pure triacylglycerols and a compositionally less complex plant oil (olive oil) has allowed us to begin to assess the likely contributions of microbial lipids to

the degraded acyl lipid profiles commonly seen in archaeological pottery. We have observed the appearance of fatty acids in the olive oil residue following 95 days of decay which were not present in fresh olive oil. The components showed similar characteristics to the minor components seen in the pure tristearin and triolein decay experiments which were thought to represent the deposition of bacterial fatty acids in potsherds. However, relative to the quantity of lipid originally present, the abundance of these components was minor. The original character of the lipid profile was clearly recognisable throughout the decay experiment, despite changes in the relative proportions of the mono-, di-, triacylglycerols and free fatty acids.

The rate of decay of olive oil proceeded more slowly than the decay of milk and is attributed partly to the numerous strains of bacteria present in unpasteurised milk and the activity of lipases which hydrolyse glycerides. Bacteria responsible for the souring of milk include *Streptococcus thermophilus*, *Lactobacillus bulgaricus* and *L. acidophilus*. These micro-organisms are found naturally in milk but can be killed by boiling. Ethnographic evidence from Bulgaria describes how milk is simmered in a pot to reduce its volume to one third in yoghurt making to kill unwanted bacteria (Ryder, 1983a). Milk also contains lipoxygenases (LOX), regio- and stereo-specific enzymes which react with free acids more readily than bound lipid to produce hydroperoxides (Hamilton *et al.*, 1997). Olive oil is less complex than milk but also contains LOX which would contribute to decay.

The free α,ω-dicarboxylic acids present in the olive oil decay experiment are formed by mechanisms known to be associated with the oxidation of unsaturated fatty acids (Simic *et al.*, 1992), although the exact pathways involved in their formation are not completely understood. Further evidence for oxidative decay has come from the presence of hydroxyoctadecenoic acids in the degraded olive oil residues. Hydroxy fatty acids have been reported in micro-organisms (e.g. Ratledge, 1988), however, due to the presence of numerous positional isomers of these oxyfunctional components in the olive oil, they more likely derive from the autoxidation of unsaturated fatty acids rather than arising through microbial action. The mechanism of $C_{18:1}$ autoxidation as described by Frankel (1998) involves hydrogen abstraction at the allylic carbon-8 and carbon-11 to produce two delocalised three-carbon allylic radicals, which when attacked at the end-carbon position

by oxygen, produce a mixture of four allylic hydroperoxides containing OOH groups on carbons 8, 9, 10 and 11 which were subsequently reduced to form the corresponding hydroxyacids. Since 8- and 11-hydroxyacids are not formed as a result of singlet oxygen reacting directly with the double bond, the oxidation mechanism occurring during the decay of olive oil in the laboratory most likely involves hydroperoxide formation *via* free radical autoxidation. The diacids and hydroxyacids previously identified in the Neolithic potsherds from Chalain (Regert *et al.*, 1998) were only present in the bound lipid fraction, indicating that due to the reactive nature of these oxidised fatty acid moieties, they are not preserved for very long as free lipids, however, where present they do provide an indicator that an abundance of unsaturated fatty acid moieties were originally present in the residue. Decay experiments conducted under a range of controlled incubation conditions may enable the exact conditions required for the formation, and importantly, the mechanism of preservation of fatty acid oxidation products to be determined. The results indicate that hydrolysis was significant in initiating the decay of the olive oil, significantly altering the overall distribution of lipid components, followed by a combination of β-and autoxidation which resulted in the rapid depletion of free fatty acids and formation of fatty acid oxidation products.

Based on the absence of characteristic marker compounds in the solvent extracts of the standard compounds, triolein and tristearin, there was no evidence for microbial activity in the sherds at the beginning of the experiments but the identification of minor abundances of straight-chain $C_{14:0}$ to $C_{18:0}$ (inclusive), $C_{18:1}$ and branched-chain $C_{15:0}$ and $C_{17:0}$ components after 57 days of incubation clearly indicates that microbes had been active in the potsherds and were likely responsible for a range of compositional changes seen in the added lipids. The possibility of migration of lipids from the compost to the sherds can be ruled out from the results of control experiments involving the incubation of buried sherds without added lipid; no significant lipid extract was obtained from any such experiments. This supports the findings of Heron *et al.* (1991). The branched-chain $C_{15:0}$ and $C_{17:0}$ fatty acids are well-known as bacterial markers (Gillan *et al.*, 1984; Goossens *et al.*, 1986; Marty *et al.*, 1996) produced by G^+ bacteria (Paul and Clark, 1996) and $C_{16:1}$ and $C_{18:1}$ are also present in high concentration in bacteria (Marty *et al.*, 1996) compared with higher organisms.

The processes of β-oxidation and reduction have been suggested as being involved in the formation of $C_{16:0}$ from free $C_{18:1}$ (den Dooren de Jong, 1961); however, a more likely origin is from microbial biomass. An accumulation of $C_{16:0}$ would be unlikely to occur unless the system was nutrient limited, since the step-wise reactions involved in β-oxidation are unlikely to halt after just one cycle. $C_{14:0}$ is known to occur as a minor component in some bacteria (Goossens *et al.*, 1986), and a review of fungal fatty acids by Lösel (1988) has shown that $C_{14:0}$ may be regarded as one of the principal bacterial fatty acids.

The relative abundance of free $C_{18:1}$ at the start of the lamb decay experiment may be a result of this component being preferentially hydrolysed from the intact triacylglycerols. The reduction in the abundance of $C_{18:1}$ fatty acids through the lamb fat decay experiment may have resulted from bacterial reworking or oxidation. The bacterial contribution of lipids can be inferred by consideration of the stable carbon isotope ratios of the individual fatty acids during the course of decay. Figure 7.24 shows that the $\delta^{13}C$ values of the $C_{18:1}$ fatty acid are very similar to the $C_{16:0}$ at T0 but less depleted than the $C_{18:0}$ by approximately 2‰. The $\delta^{13}C$ value of the $C_{18:1}$ is even less depleted (by approximately 1 to 2‰) by the end of 1300 days of decay under both anoxic and autoclaved oxic conditions. The same pattern is seen in the $\delta^{13}C$ values of the $C_{14:0}$ fatty acid. Minor variation may be due to the relatively low intensity of the $C_{14:0}$ in the decayed fat and high abundance of the $C_{18:1}$ in the fresh fat which may increase the error of $\delta^{13}C$ measurements made by GC-C-IRMS. It is possible that microbial lipids have contributed to the less depleted values for these fatty acids, especially since $C_{14:0}$ acid is regarded as one of the major bacterially-derived acids. However, it is reassuring that even after almost complete depletion of lipid in the lamb decay experiments, the $\delta^{13}C$ values of the $C_{16:0}$ and $C_{18:0}$ fatty acids are unaffected. Furthermore, there is no evidence from this data for β-oxidation and reduction of the $C_{18:1}$ fatty acid during decay as suggested by den Dooren de Jong (1961), since we would surely see this reflected in the $\delta^{13}C$ value of the $C_{16:0}$ fatty acid.

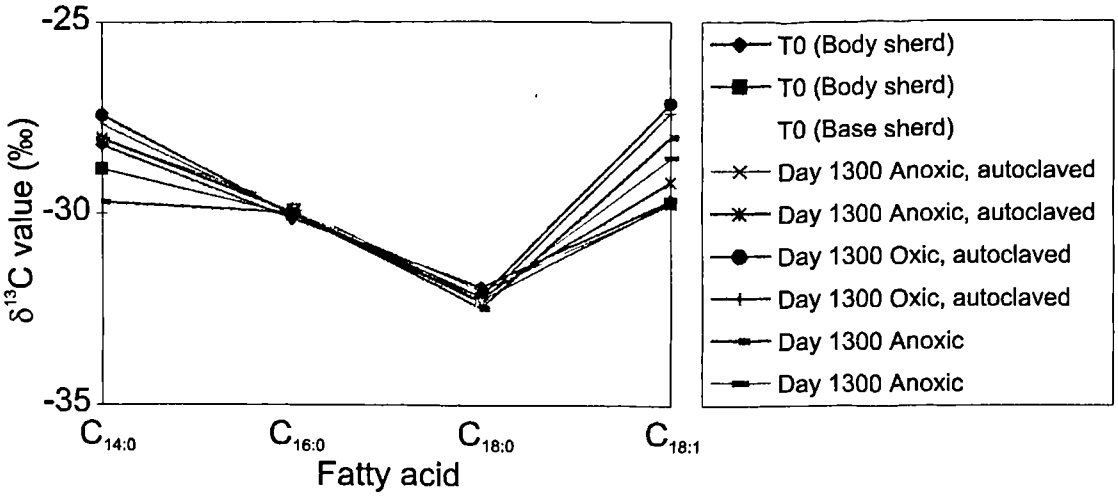


Figure 7.24 Stable carbon isotope data obtained for the saturated fatty acids present in the hydrolysed lamb fat at T0 and in lamb fat following 1300 days of oxic and anoxic decay absorbed in ceramic sherds.

It is likely that in archaeological lipid residues the processes of decay would already have been initialised prior to burial due to normal vessel use, e.g. by oxidative, hydrolytic and thermal decomposition processes (Charters *et al.*, 1995; Charters, 1996). Indeed, alteration of the original lipid character prior to burial is evidenced by the presence of mid-chain ketones in numerous vessels where animal fats have been heated to temperatures in excess of 300°C (Evershed *et al.*, 1995b; Raven *et al.*, 1997). Moreover, studies of ethnographic vessels that have never been buried have shown patterns of hydrolytic change in acyl lipids analogous to those seen in buried potsherds (Charters, 1996; Evershed *et al.*, 1997a, b). The decay experiments described herein show how similar patterns of degradation occur for acyl lipids of widely varying origin, and furthermore, that although the HTGC profiles of degraded lipid can be readily related to the intact fats and oils from which they derive, various criteria need to be taken into consideration when making assignments. Reliance on one criterion such as the distribution of intact triacylglycerols may lead to misinterpretation due to the preferential diagenesis of diagnostic components as seen in the *case of dairy fats.*

As a result of experimental work carried out by Charters (1996) and as part of this study the high preservation potential of leaf wax components compared with acyl lipids derived from animal fats has been established. It is therefore surprising that we do not see leafy vegetable markers (such as the alkanes and ketones found in *Brassica* sp.) with more

frequency in prehistoric vessels. This is a general observation based upon extractions of a substantial number of sherds in our laboratory dating from the Iron Age to the early Neolithic. Indeed, to date no evidence of higher plant material has been found in pottery residues prior to the Iron Age which is surprising since long-chain alkyl components are preserved with such high frequency in the medieval vessels from West Cotton (Charters, 1996). The absence of these residues in early pottery would appear to be real and, assuming that prehistoric man was utilising plant materials, the negative evidence suggests that pottery vessels were not being used to boil leafy vegetables.

7.8 Conclusions

This experimental work has provided substantial new data which have indicated the effects of decay on the distributions of lipid components in natural commodities and has enabled an assessment of the contribution of microbial lipids to decay, thereby improving our general understanding of diagenetically altered residues with consequence for future analyses and interpretations on the basis of the following observations:

1. Available lipid will be readily and rapidly degraded by a combination of hydrolytic, oxidative and enzymatic processes, with the rate determining step appearing to be the cleavage of the initial fatty acid from the intact triacylglycerol.

2. The decay of olive oil under laboratory conditions has been shown to proceed (at least in part) *via* free radical autoxidation as evidenced by the presence of 8- and 11-$C_{18:1}$ hydroxyacids.

3. Although a large proportion of the lipid originally present at T0 had been experimentally degraded in the laboratory, the remaining lipid comprising plant oils, leaf waxes and adipose fats was afforded sufficient protection such that it reflected the original lipid profile.

4. Direct heat at the base of vessels appeared to have significantly altered the original *cis*-configured $C_{18:1}$ fatty acid distributions, showing the relatively high susceptibility of

these isomers to thermal decomposition. However, the ratio of *trans* acids were found to be similar before and after laboratory decay due to their relatively high stability, providing a reliable criterion upon which to characterise degraded ruminant fats.

5. It has been confirmed experimentally that the preferential loss of the shorter-chain free fatty acids would contribute to our failure to distinguish degraded archaeological milk fats from degraded adipose fats in archaeological pottery.

6. The extent of incorporation of bacterial and fungal markers in decay experiments was low (<2%), thus representing a minor, and insignificant, contribution to the overall lipid profile even in the case of highly degraded material (>90% consumed), and fully legitimizing the use of absorbed lipids in archaeological investigations.

7. The original stable carbon isotope signals of the major saturated fatty acids have been shown to be diagenetically robust, thus validating their use in characterising the origins of fats. Even after long-term incubation the $\delta^{13}C$ values still reflected the differences in the physiological and metabolic processes involved in the original formation of the lipid components.

These preliminary results are extremely encouraging, validating the use of acyl lipids preserved in ancient ceramics as a source of archaeological information and providing new insights into the processes by which the original lipid profiles of fats and oils are diagenetically transformed within the microstructure of a ceramic sherd.

CHAPTER 8
Overview

8.1 The use of chemical criteria to distinguish between animal fats of different species origin.

8.1.1 Summary of the isotopic and distributional data used to characterise fats from the major domesticated species.

8.1.1.1 Ruminant adipose fats

Ovine fats are characterised by:

i) a higher proportion of the $C_{18:0}$ than $C_{16:0}$ fatty acid;

ii) $\delta^{13}C$ values ranging between -28‰ to -29.3‰ for the $C_{16:0}$ fatty acid, and between -29.6‰ and -30.9‰ for the $C_{18:0}$ fatty acid;

iii) a high abundance of the *trans*-Δ^{11} $C_{18:1}$ fatty acid and a higher *trans*- than *cis*-$\Delta^{11/10}$ ratio;

iv) intact triacylglycerols ranging between C_{46} to C_{54} with relatively abundant saturated C_{54} and C_{52} components (in the saturated fraction of intact triacylglycerols);

v) diacylglycerols ranging between C_{30} and C_{36}.

Bovine adipose fats are distinguishable from ovine adipose fats by:

i) a higher proportion of the $C_{16:0}$ than $C_{18:0}$ fatty acid;

ii) a higher relative abundance of the C_{50} triacylglycerol component than in ovine adipose fats (in the saturated fraction of intact triacylglycerols);

iii) $\delta^{13}C$ values which are more depleted than ovine fats by ca. 0.6‰ and 1.2‰ for the $C_{16:0}$ and $C_{18:0}$ fatty acids, respectively;

iv) a lower abundance of the *trans*-Δ^{11} isomer in cow adipose compared to sheep adipose.

8.1.1.2 Dairy fats

Dairy fats can be distinguished by:

i) $\delta^{13}C$ values ranging between -27.3‰ to -29.8‰ for the $C_{16:0}$ fatty acid and between -31.9‰ and -33.7‰ for the $C_{18:0}$ fatty acid;

ii) diacylglycerol distributions ranging between C_{28} to $C_{30/36}$; iii) broad triacylglycerol distributions as discussed in Sections 4.2.9 and 6.5.2.1;

iv) a relatively high abundance of the $C_{14:0}$ fatty acid, as indicated by the $C_{14:0}$:$C_{17:0}$ ratio;

v) a high abundance of the *trans*-Δ^{11} $C_{18:1}$ fatty acid and a higher *trans* than *cis* $\Delta^{11/10}$ ratio.

Degraded dairy fats are also characterised by an abundance of shorter-chain length free fatty acids, ranging between $C_{8:0}$ to $C_{12:0}$, which are present due to the hydrolysis of lower carbon number triacylglycerols. However, this is infrequently useful as an indicator of archaeological dairy fats since these fatty acids are highly susceptible to loss by dissolution in groundwaters. Bovine dairy fats are tentatively distinguished from ovine dairy fats on the basis of the ratio of $C_{16:0}$:$C_{18:0}$ fatty acids.

8.1.1.3 Non-ruminant fats

Porcine fats are identified on the basis of:

i) $\delta^{13}C$ values ranging between -26‰ to -27‰ for the $C_{16:0}$ fatty acid and between -24.7‰ and -25.6‰ for the $C_{18:0}$ fatty acid;

ii) the absence or very low abundance of branched-chain and odd-carbon number fatty acids, indicated by a high $C_{14:0}$:$C_{17:0}$ ratio;

iii) a higher abundance of the $C_{16:0}$ than the $C_{18:0}$ fatty acid;

iv) a low abundance or absence of *trans*-configured $C_{18:1}$ components;

v) a narrow diacylglycerol distribution ranging between C_{32} to C_{36}, often with a distinctively high abundance of the C_{34} component compared with the C_{32} and C_{36};

vi) a distinctively narrow distribution of triacylglycerols as discussed in Section 5.2.6.

Other animal species, including horse and poultry are not easily recognisable due to the similarity of their fat composition to the major domesticates as already discussed. However, examples of well preserved horse fats should be readily recognisable by:

i) the distinctively low abundance of the $C_{16:0}$ fatty acid in relation to the $C_{18:0}$ component;

ii) a relatively high abundance of the $C_{14:0}$ fatty acid;

iii) low abundances of branched-chain $C_{15:0}$ and $C_{17:0}$ components;

iv) distributions of $C_{18:1}$ positional isomers similar to non-ruminants, including low abundances of the *trans*-Δ^{11} component.

Deer adipose is almost identical in chemical character to sheep adipose, and poultry fats are very similar to porcine fats, but with somewhat different distributions of intact triacylglycerols.

8.1.1.4 Mixtures of fats

Mixtures of non-ruminant and ruminant fats can be identified on the basis of intermediate $\delta^{13}C$ values and distributions of $C_{18:1}$ positional isomers which indicate a contribution from different fat types. Proportions of different fats in the same vessel can be estimated according to the position on the theoretical mixing curve, although their stable carbon isotope composition may be variable according to the diet of the animal.

Due to the varying states of decay in which archaeological fats are found, the whole range of chemical criteria are not always applicable in all of the archaeological fats, however, a combination of several of the more diagnostic criteria gives a good indication of the origin of the remnant fat. Figure 8.1 summarises the distributional characteristics of the reference fats and olive oil.

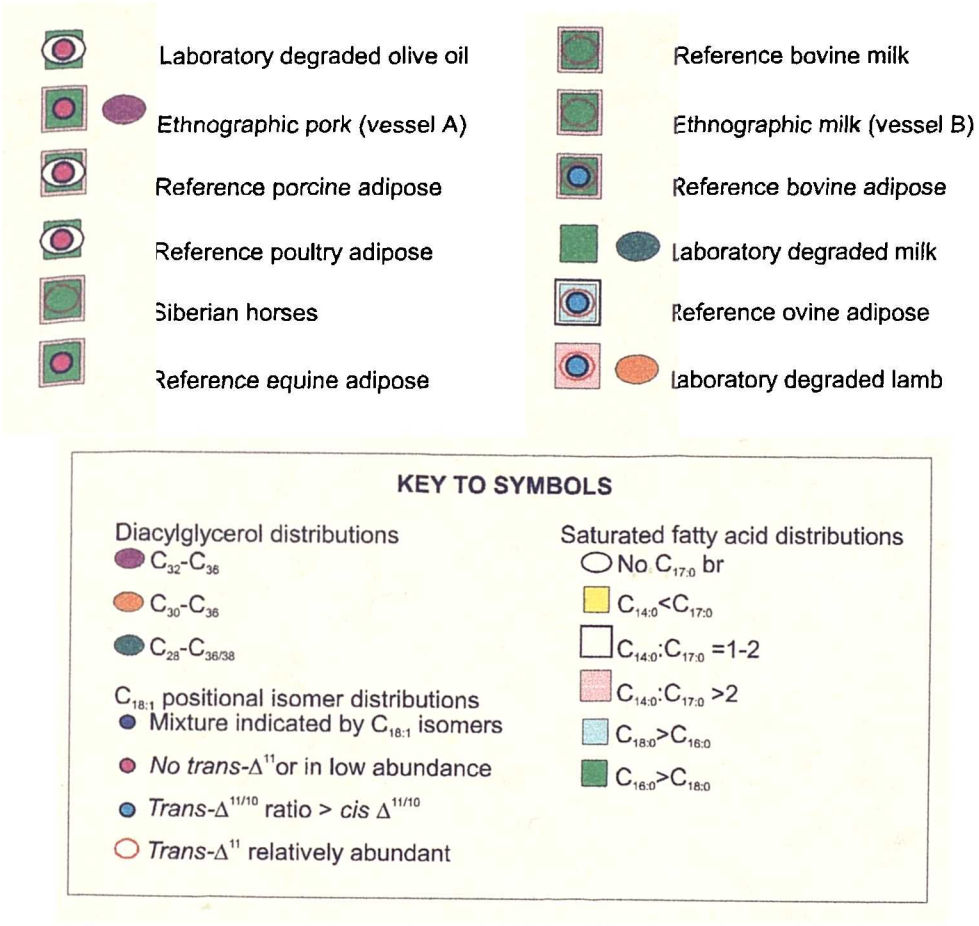


Figure 8.1 Summary characteristics of the lipid distributions of reference fats and olive oil. Symbols are explained in the key and triacylglycerol distributions detailed in Chapter 5. Combinations of these symbols are used to denote the chemical characteristics of lipid distributions in archaeological fats plotted in Figures 8.2, 8.3 and 8.6 to 8.19.

Based on these criteria, laboratory degraded olive oil appears very similar to porcine fats but ethnographic examples and the TLC fractionation suggests that degraded olive oil should be readily distinguishable on the basis of the distinctive distribution of intact triacylglycerols and by the high $C_{16:0}$:$C_{18:0}$ ratio and high abundance of oleic acid, even in degraded fats.

8.1.2 Use of ethnographic residues as reference material

Ethnographic vessels of known domestic function were examined in order to make comparisons between degraded and fresh reference fats. Two of the vessels (A and F) are known to have been used for the preparation of pork dishes, while two others (B and G) were used to for the storage of milk. The residues from ethnographic vessels A and F were found to be very similar to the reference pork fat, with high $C_{16:0}/C_{18:0}$ ratios and highly abundant $C_{18:1}$ reflecting the high unsaturation of pork fat. The $C_{14:0}/C_{17:0}$ fatty acid ratio for the ethnographic pork residue in vessel A was 3.3 which compared well to the ratio in the reference fat of 3.97. The same ratio in vessel F was 7.37, reflecting the very low abundance of $C_{17:0}$ usually observed in pork fat. Furthermore, the $C_{18:1}$ *cis*-Δ^{11} component was present in high abundance, which has been shown to be characteristic of non-ruminant fats. The only anomaly between the ethnographic and reference pork fat was the abundance of the isomers in positions Δ^{10}, Δ^{12}, Δ^{13}, Δ^{14} and Δ^{15} which are absent, or present in very low abundance, in the reference porcine fat. The accumulation of exogenous dietary fatty acids directly into adipose tissue of typical omnivores explains the appearance of branched-chain and *trans*-configured $C_{18:1}$ components in the ethnographic pig fats. These are likely to derive from dairy slops or ruminant meat/fat scraps in the animal's diet. This accumulation of branched-chain components has also been shown in human depot fat (Shorland *et al.*, 1969; Jacob and Grimmer, 1967). The other ingredients used in the preparation of the pork dishes in vessels A and F, i.e. tomato sauce, paprika, oregano, onions, bahari (spice), salt and pepper left no apparent lipid residue, indicating the problem of detecting other minor ingredients used in antiquity through the analysis of solvent-extractable lipid residues, and that the absence of chemical evidence does not necessarily mean they were not utilised.

The presence of the short-chain $C_{12:0}$ component, the abundance of odd-carbon number and branched-chain fatty acids and the distribution of positional isomers is consistent with the

storage or processing of milk or cheese in vessels B and G. The reference dairy fats are characterised by a greater abundance of the *cis*-Δ^{11} than that seen in the extract from vessel B, which is probably due to the greater susceptibility of the *cis*-configured isomers to decay. The ratio of $C_{16:0}$ and $C_{18:0}$ fatty acids is consistent with that seen in the reference cows' milk. This is strongly supported by the ratios of $C_{18:1}$ isomers in the Δ^{10} and Δ^{11} positions. The $C_{12:0}$ fatty acid is present, however, there is a lack of fatty acids with < 10 carbon atoms which are also seen in reference milk fat, but this reflects the extent of the decay which has occurred. The effect of decay is also seen in the overall low abundance of the monounsaturated C_{18} components and also the preferential loss of the $C_{18:1}$ isomers of the *cis* form.

8.2 Interpretations of the chemical characteristics of archaeological fats

8.2.1 Sites with well-documented faunal assemblages

8.2.1.1 West Cotton (Late Saxon/early medieval)

This domestic assemblage has yielded abundant animal fat and leafy vegetable residues, and was chosen for analysis partly because of the excellent preservation of the faunal assemblage from the site and the plentiful and well-documented archaeological data. The distributions of $C_{14:0}$, $C_{16:0}$ and $C_{18:0}$ fatty acids and $C_{18:1}$ positional isomers in individual samples from West Cotton shown in Figure 8.2 and the combination of isotopic data, and di- and triacylglycerol distributions shown in Figure 8.3 clearly enable distinctions to be drawn between remnant fats from this assemblage. Consideration of the range of data enabled several different groups of fats to be recognised. Arbitrary rings have been drawn around these groups in order to indicate the range of $\delta^{13}C$ values for fats with similar distributional characteristics and indicating variations between the mean $\delta^{13}C$ data from modern reference and archaeological fats (Fig. 8.3).

The combination of data obtained by positional isomer analysis, di- and triacylglycerol distributional analysis and $\delta^{13}C$ measurements have shown unambiguously that the majority of adipose fats from West Cotton derive from ruminant fats. The abundance of the *trans*-Δ^{11} components present and the higher abundance of the $C_{16:0}$ than the $C_{18:0}$ fatty acid

indicates more specifically that they derive from an ovine origin. These data correlate well with the high proportion of sheep bone excavated from the site.

The identification of dairy fats in several of the West Cotton samples through the analysis of stable carbon isotope ratios is supported by triacylglycerol distributions and the relatively high abundance of the $C_{14:0}$ fatty acid indicative of dairy fats. Characteristic $C_{18:1}$ positional and geometric isomer distributions, narrow ranges of di- and triacylglycerol distributions and the absence of branched-chain fatty acids indicated the presence of non-ruminant fats. No bovine adipose fats have been unambiguously identified based upon relative abundances of intact triacylglycerols which would suggest that although cattle were present at West Cotton, as established by faunal studies, they were being kept for milk production (and possibly traction) rather than as beef animals, whereas sheep were kept for their meat and milk. The extracts identified as possible mixtures of fats comprise distributions with characteristics of both sheep and porcine adipose. Overall, these data correlate with the suggestion based upon faunal evidence that there was a bias towards the use of sheep at the site over other domesticates.

Consideration of all the distributional and stable carbon isotope data has shown that the remnant fats from West Cotton comprise 9 dairy fats, including RP22, 86, 72, 91, 30, 94, 60, 61 and WC30, with 3 samples tentatively identified as bovine dairy (RP22, 86 and 91) and the remainder from an ovine origin. Nine extracts have been identified as (predominantly) ovine adipose, including RP81, 16, 89, 7, 13, 53, 71, 93 and 87. Nine extracts are believed to represent mixtures of ruminant and non-ruminant fats, including RP85, 50, 6, 82, 83, 78, 28, 73 and 2, comprising varying proportions of the different fats and exhibiting characteristics of both porcine and ovine fats. Three remnant fats (RP10, 4 and 88) derive predominantly from a non-ruminant (e.g. porcine) origin. A few anomalies exist, including RP82 and RP73, for which the isotopic data indicate mixtures of ruminant and non-ruminant fats, but the di- and triacylglycerol distributions correspond with ruminant dairy fats. These remnant fats may represent mixtures of non-ruminant and dairy fats or derive from dairy fats exhibiting more enriched $\delta^{13}C$ values, as seen in the analysis of commercial milk fats from animals raised on ^{13}C-enriched diets.

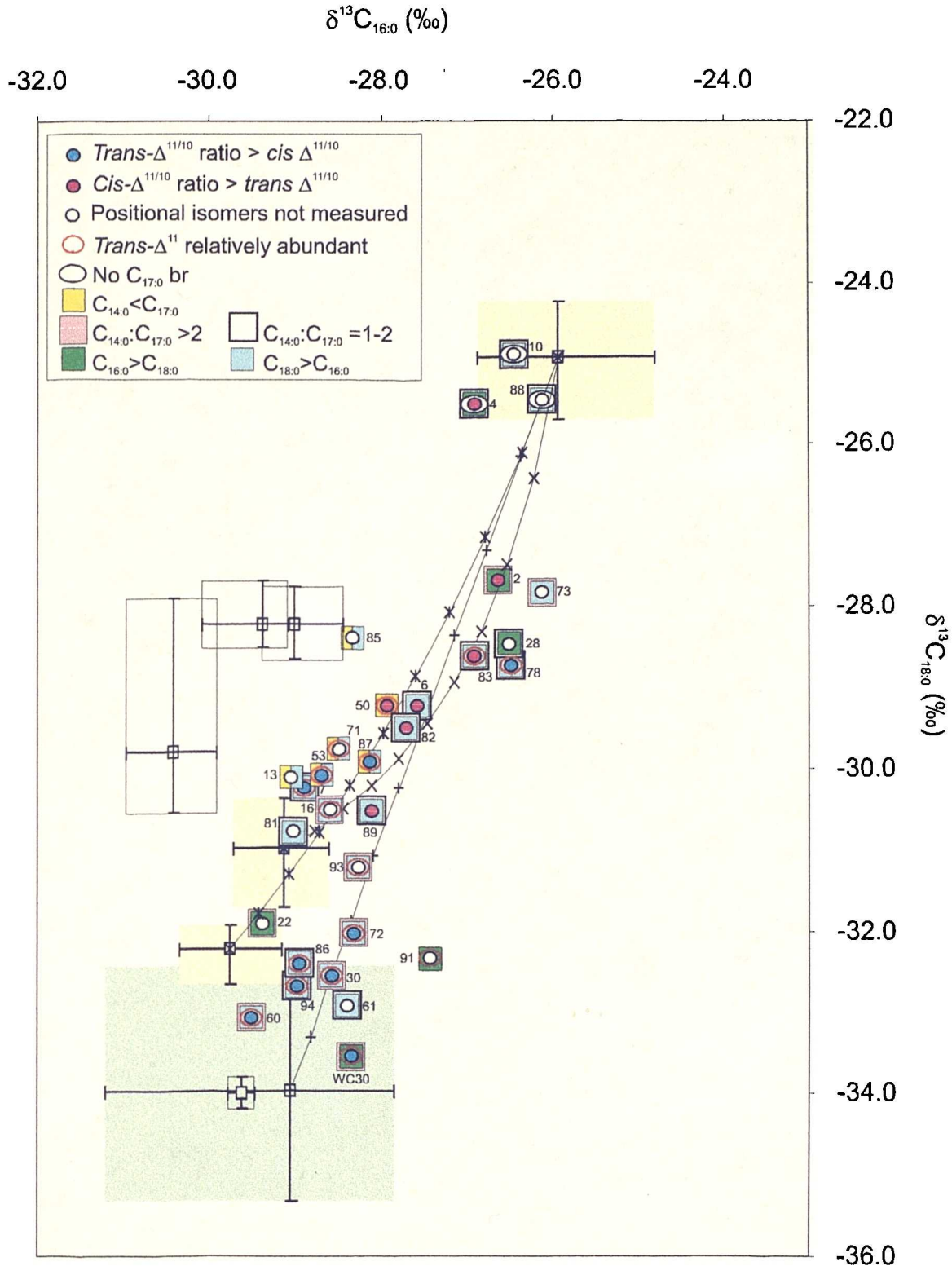


Figure 8.2 Distributions of fatty acids, including saturated $C_{14:0}$, $C_{16:0}$, $C_{17:0}$ and $C_{18:0}$ components and $C_{18:1}$ positional and geometric isomers, correlated with $\delta^{13}C$ values in remnant fats from the West Cotton assemblage.

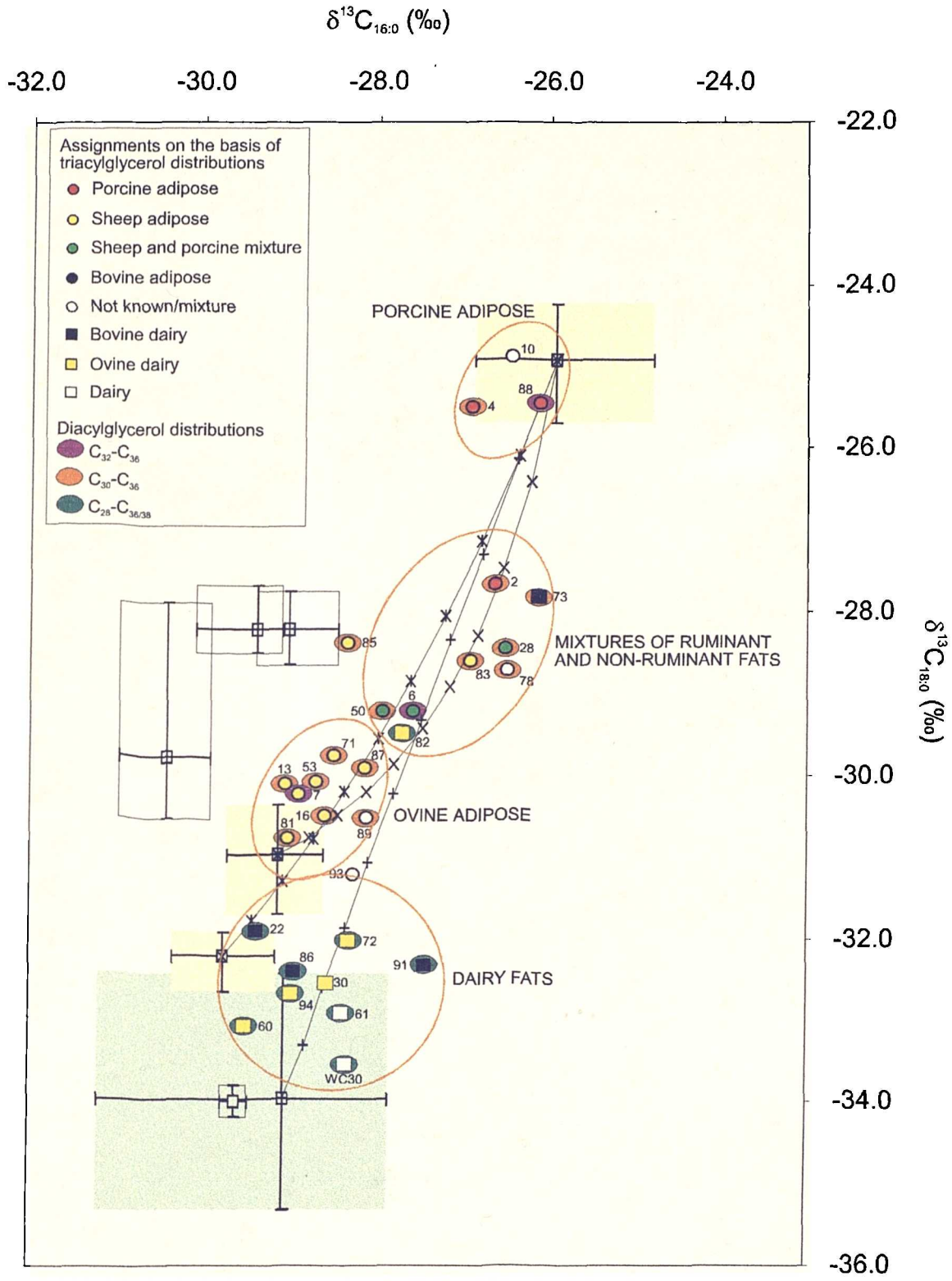


Figure 8.3 Distributions of di- and triacylglycerols in West Cotton extracts correlated with $\delta^{13}C$ values. Groups of archaeological fats distinguished by similar chemical characteristics have been indicated by the arbitrary rings.

The trends in $\delta^{13}C$ data for the remnant fats from West Cotton are strongly supported by the distributions of intact triacylglycerols in the extracts. The remnant fats displaying the heaviest $\delta^{13}C$ values for their C_{16} and C_{18} fatty acids (e.g. RP4 and RP88) corresponding to the non-ruminant reference fats comprise triacylglycerols in a distribution which closely resembles the distribution characteristic of fresh porcine adipose fat [Fig. 8.4 (a) and (b)]. The majority of the remnant fats with more depleted $\delta^{13}C$ values ranging between -27 to -31‰ (e.g. RP13 and RP89), display triacylglycerol distributions not dissimilar to the reference ruminant adipose fat [Fig. 8.4 (e)], whilst the distributions of triacylglycerols in those vessels yielding the lightest $\delta^{13}C$ values (ca. -31 to -34‰; e.g. WC30 and RP61) show a very close correspondence to the triacylglycerols in the laboratory degraded milk fat [Fig. 8.4 (h)].

In Figure 8.3, groups of archaeological fats distinguished by a range of similar chemical characteristics have been indicated by arbitrary rings. The fact that the $\delta^{13}C$ values of fats within different groups cluster together is extremely encouraging for the use of stable isotope data in the analysis of archaeological lipids and indicates that the fat composition of animals from the same species raised at West Cotton does not vary significantly. However, in general the mean $\delta^{13}C$ values for the remnant ruminant fats from West Cotton are more enriched than the means of the data for the modern reference fats in both the $C_{16:0}$ and $C_{18:0}$ fatty acids by ca. 0.6‰ for the ovine adipose and 1.5‰ for dairy fats, even after taking into account the 1.2‰ change in $\delta^{13}C$ values of atmospheric CO_2 since the Industrial Revolution. The fatty acids in the archaeological non-ruminant fats from West Cotton exhibit very similar $\delta^{13}C$ values to the reference pig fats, with the mean differing by only ca. -0.3‰ compared with the modern reference pig fats.

Extremely useful data have been obtained from the analyses of positional and geometric isomer distributions in the West Cotton extracts, although it has been essential to be able to recognise the different effects of decay on the distributions of the *cis*- and *trans*-configured components in order to enable correct interpretations of the archaeological data. In Figure 8.5 the distributions of $C_{18:1}$ isomers in reference pig adipose (a) are compared with pork fat from an ethnographic vessel (b) and the archaeological extract from RP4 (c). The reference pig fat and the ethnographic pork residue comprise predominantly the *cis*-Δ^9 and *cis*-Δ^{11},

although there are other minor isomers present in the ethnographic fat probably derived from the diet of the animal. The absence of both the *cis*- and *trans*-Δ^{11} components in RP4 is indicative of degraded non-ruminant (e.g. porcine) fat. The distributions of isomers in reference sheep adipose are compared with the archaeological extract from RP16 [Fig. 8.5 (e)]. Ruminant adipose comprises a high abundance of the *trans*-Δ^{11} component with minor amounts of the corresponding *cis*-isomer. In the archaeological fat the *trans* isomer is still predominant, even though the proportion of the *cis*-Δ^{9} component has been dramatically reduced. The abundance of minor positional and geometric isomers are also characteristic of ruminant fats.

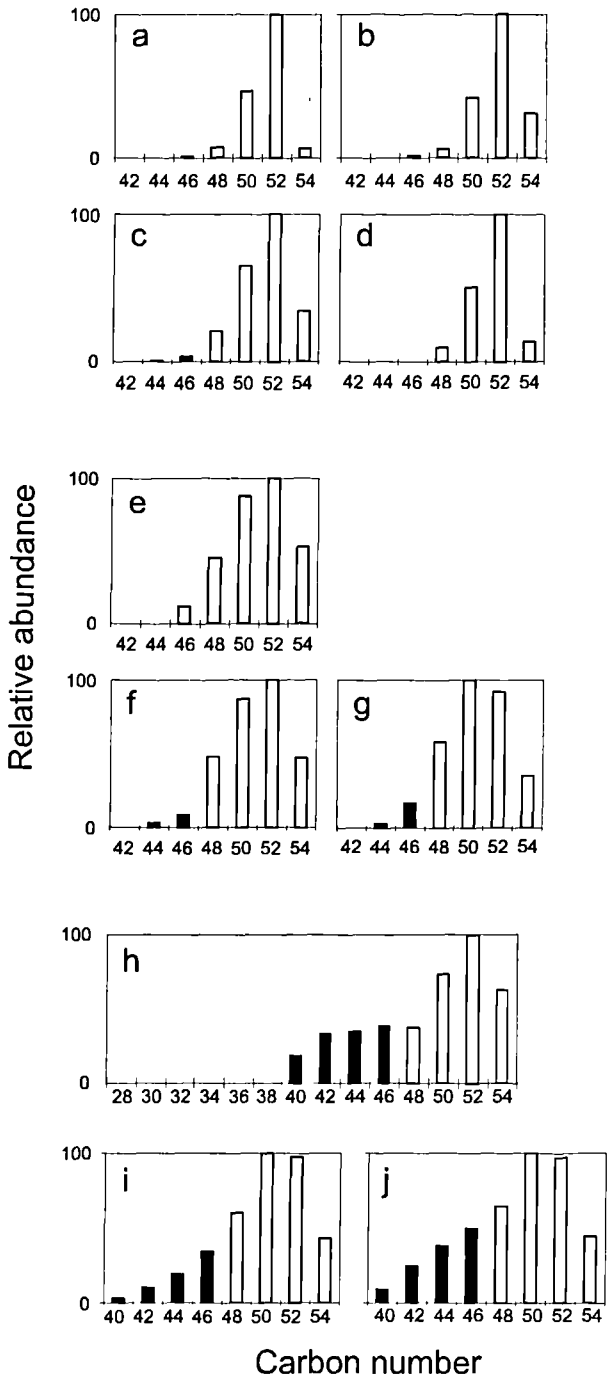


Figure 8.4 Distributions of intact triacylglycerols in remnant fats from West Cotton compared with distributions in reference fats. These data are wholly supportive of the distinctions made on the basis of $\delta^{13}C$ values. The fats shown are: (a) modern pork fat which have been separated using TLC to derive the saturated moieties; (b) remnant pork fat from a 45 year old ethnographic vessel; (c) archaeological fat from RP4; (d) archaeological fat from RP88; (e) modern lamb fat which has undergone laboratory decay; (f) archaeological fat from RP13; (g) archaeological fat from RP89; (h) milk fat which has been degraded in the laboratory for 90 days; (i) archaeological fat from WC30, and (j) archaeological fat from RP61.

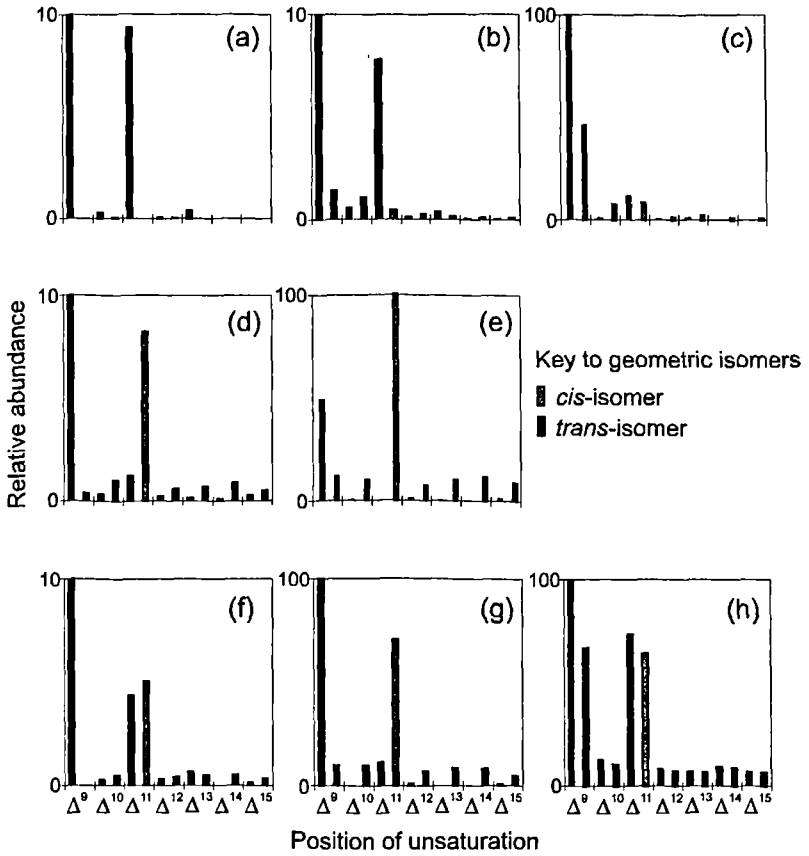


Figure 8.5 Distributions of positional and geometric isomers of the monounsaturated C_{18} fatty acid from modern reference fats, extracts from ethnographic vessels and archaeological pottery extracts from the West Cotton assemblage, including: modern reference pig adipose (a); ethnographic pork (b); RP4 (c); modern reference sheep adipose (d); RP16 (e); modern reference cows' milk (f); ethnographic milk (g), and WC30 (h). The diagnostic isomers are those highlighted in blue and green. Note the scale is different for the modern reference and the archaeological fats due to the predominance of the *cis*-Δ^9 component in the modern fats.

Figure 8.5 (f) shows the isomers of $C_{18:1}$ present in reference milk fat which are dominated by the *cis*-Δ^9, *cis*-Δ^{11} and *trans*-Δ^{11} components. The Δ^{11} isomers are present in approximately equal proportions in the reference fats, however, the *cis*-Δ^{11} has been lost from the ethnographic milk fat (g) due to its higher susceptibility to decay and the proportion of *cis*-Δ^9 has been greatly reduced. Figure 8.5 (h) shows the $C_{18:1}$ isomers present in the extract from WC30, a medieval 'top hat' pot from West Cotton. The remnant fat from this vessel had been previously tentatively identified as a dairy fat on account of the high abundance of short-chain fatty acid components in the residue. The abundance of $C_{18:1}$ *cis* isomers and short-chain saturated fatty acids present in WC30 reflects the excellent preservation afforded to this extract. The presence of both the *cis*-Δ^{11}

and *trans*-Δ^{11} components supports the assignment that this residue is derived from a dairy fat.

From the samples analysed the relative proportions of the different commodities processed are 10% porcine, 30% ovine adipose and 30% dairy. The remaining 30% appeared to comprise mixtures of ruminant and non-ruminant fats. This gives a frequency of 25% porcine fats to 75% ruminant adipose fats processed in the vessels analysed, although this does not take into account the vessels containing mixtures of fats which in general appear to be comprised of a higher proportion of ovine than porcine fat (as indicated by their position on the mixing curve). The proportions of the major domesticates farmed at West Cotton have been estimated from the faunal remains by Albarella and Davis (1994), indicate that in the early medieval period 80% of the animals were ruminant (ovine and bovine) whilst 20% were porcine. Our data collected over several periods in the history of West Cotton show a similar proportion of ruminant to non-ruminant animals, however, a larger data set from each archaeological period would be required to make more accurate comparisons. The large number of immature pigs identified in the faunal assemblage at West Cotton supports the use of these animals for meat or lard production.

The number of vessels containing ruminant fats well out-number those used in association with non-ruminant fats, although this may be partly a reflection of cooking methods, for example pigs may have been more commonly prepared by spit roasting rather than by boiling or roasting in a pot. As noted in Section 2.2.1.1, the butchery marks observed on both the cattle and horse bone at West Cotton are strong indicators that their meat was being utilised, and chicken, goose, duck and pigeon are believed to have served as a source of meat and fat, although none of the remnant fats have been identified unambiguously as deriving from these species at West Cotton. It is not clear from the isotopic data alone whether any of the remnant fats derive from horse, chicken or goose fats. A high proportion of the archaeological fats appear to derive from ruminant milk fats. This is perhaps not unexpected since pottery vessels are ideal receptacles for liquids and can withstand high temperatures during boiling, pasteurising, etc. Consideration of the volume of milk which could have been collected at any one time would necessitate the use of numerous receptacles, e.g. skin bags, pottery vessels or wooden pales.

The similarity between the $\delta^{13}C$ values obtained for the three non-ruminant fats from West Cotton may suggest that the swine at West Cotton were panned or housed. Early Neolithic evidence also suggests that there would have been very little out-breeding to wild swine suggesting sows were brought in for the breeding season and housed over winter (Gregg, 1988). These factors would be significant in determining the isotopic signal of the tissues of the animals, as would the diet of the animals since the tissues of mono-gastric animals (e.g. pigs) are more directly affected by their diet. It is widely believed that pigs would have been encouraged to forage in woodland, e.g. for acorns and hazel nuts, which are high in carbohydrate, and would also consume a variety of domestic waste, e.g. leftovers of cheese production and crop processing, which may well have been used to fatten them up and improve the flavour of their meat, however, these data indicate that at West Cotton the diets of the animals were of relatively constant isotopic composition.

8.2.1.2 Stanwick (Iron Age /Romano-British)

The data from Stanwick also suggest the exploitation of various animal fats detected through their containment in pottery vessels. The data indicate that all of the remnant fats from Stanwick derive from ruminant fats (Figs. 8.6 and 8.7), with 6 extracts out of 19 identified as ruminant dairy fats, including ST208, 161, 160, 197, 206 body, 194 and 197. The majority of the dairy fats appear to derive from a bovine origin, whilst ST197 is more characteristic of an ovine fat. A range of vessel types, including bowls, jars and dishes were found to contain remnant dairy fats present in relatively high abundances of lipid per gram of powdered sherd, possibly indicating that they contained butter. Butter is more fatty than milk and more likely to leave a substantial residue.

The remaining 13 extracts are believed to represent adipose fats, with the majority characteristic of ovine fats. The stable carbon isotope data indicate that several of the extracts represent mixtures of ruminant and non-ruminant fats since they plot along the line of the mixing curve but the isotope composition of the fats may have been influenced by a diet more enriched in ^{13}C since in general these extracts exhibit distributional characteristics of ovine adipose fats. It should not be ruled out that a number of these adipose fats derive from a bovine source but are indistinguishable based upon lipid distributions due to diagenetic alteration.

The faunal assemblage from Stanwick comprised 11% and 6% pig during the Romano-British and Iron Age periods, respectively, and the high percentage of ruminant animals is reflected in the data, however, none of the remnant fats have been identified as having a non-ruminant (e.g. porcine) origin. The lack of chemical evidence for non-ruminant animals from Stanwick may be as a result of the unrepresentative selection of samples from the assemblage, or a reflection of the cooking methods used at the site, e.g. pork meats/fat were more commonly processed in pottery vessels than porcine meats which were cooked over a spit.

It has been suggested that sheep milk would have been more commonly utilised than cow's milk in antiquity, firstly, due to the fact that sheep milk has a higher nutritional value than goat milk or meat which in turn has a higher nutritional value than cow's milk. The nutritional value is dependant on fat content and influenced by the fact that the fat globules in goat and sheep milk are much smaller and easier to digest than cow's milk. At Stanwick the majority of dairy fats appear to be bovine, identified by the ratio of $C_{16:0}$ and $C_{18:0}$ fatty acids and the distributions of intact triacylglycerols in the remnant fats. Conversely, at West Cotton the chemical criteria indicate that the majority of dairy fats are from an ovine source. The data can be interpreted as showing that bovine animals were rarely killed for their meat and/or fat at both Stanwick and West Cotton and would have been used mainly for their milk (and also probably for traction) whereas ovines would have been exploited both for their milk and meat.

It is possible that we are interpreting the data incorrectly and that in fact the groups of fats identified as ovine adipose at both West Cotton and Stanwick actually represent bovine adipose and that some of the less depleted remnant fats characterised as 'mixtures' actually represent mainly ovine adipose fats (since in the reference data the bovine adipose are more depleted in ^{13}C than ovine adipose by ca. 1.5‰ between the means). Comparison of the ranges of $\delta^{13}C$ values for the groups of fats distinguished at West Cotton and Stanwick shows that the stable carbon isotope data are more enriched by up to 1‰ in both the $C_{16:0}$ and $C_{18:0}$ fatty acids between the ruminant fats and 'mixtures' of fats in the West Cotton extracts than in the Stanwick extracts. In addition, the lipid components in the archaeological ruminant fats are generally more enriched than the modern reference

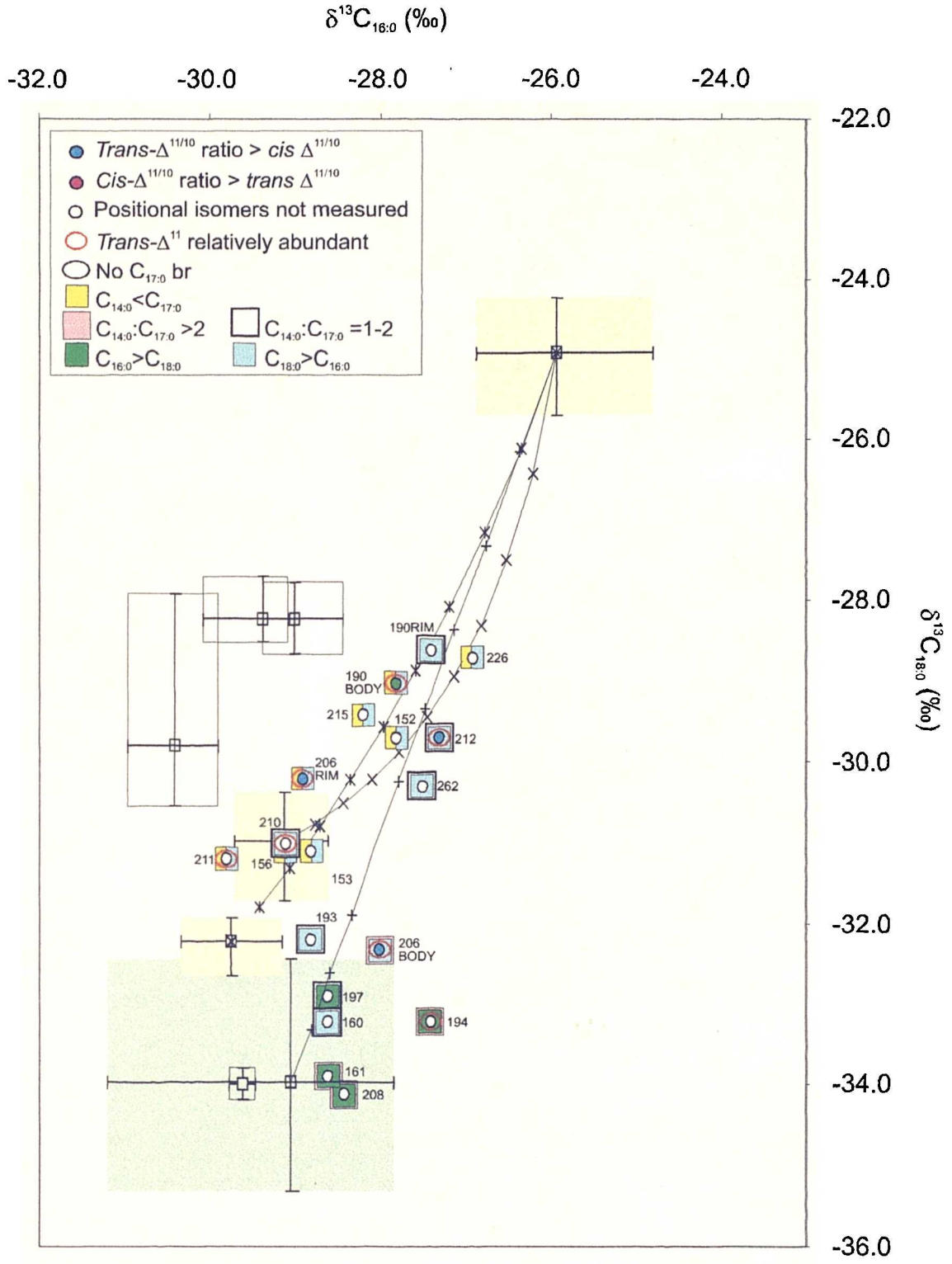


Figure 8.6 Distributions of fatty acids, including saturated $C_{14:0}$, $C_{16:0}$, $C_{17:0}$ and $C_{18:0}$ components and $C_{18:1}$ positional and geometric isomers correlated with $\delta^{13}C$ values in remnant fats from the Stanwick assemblage.

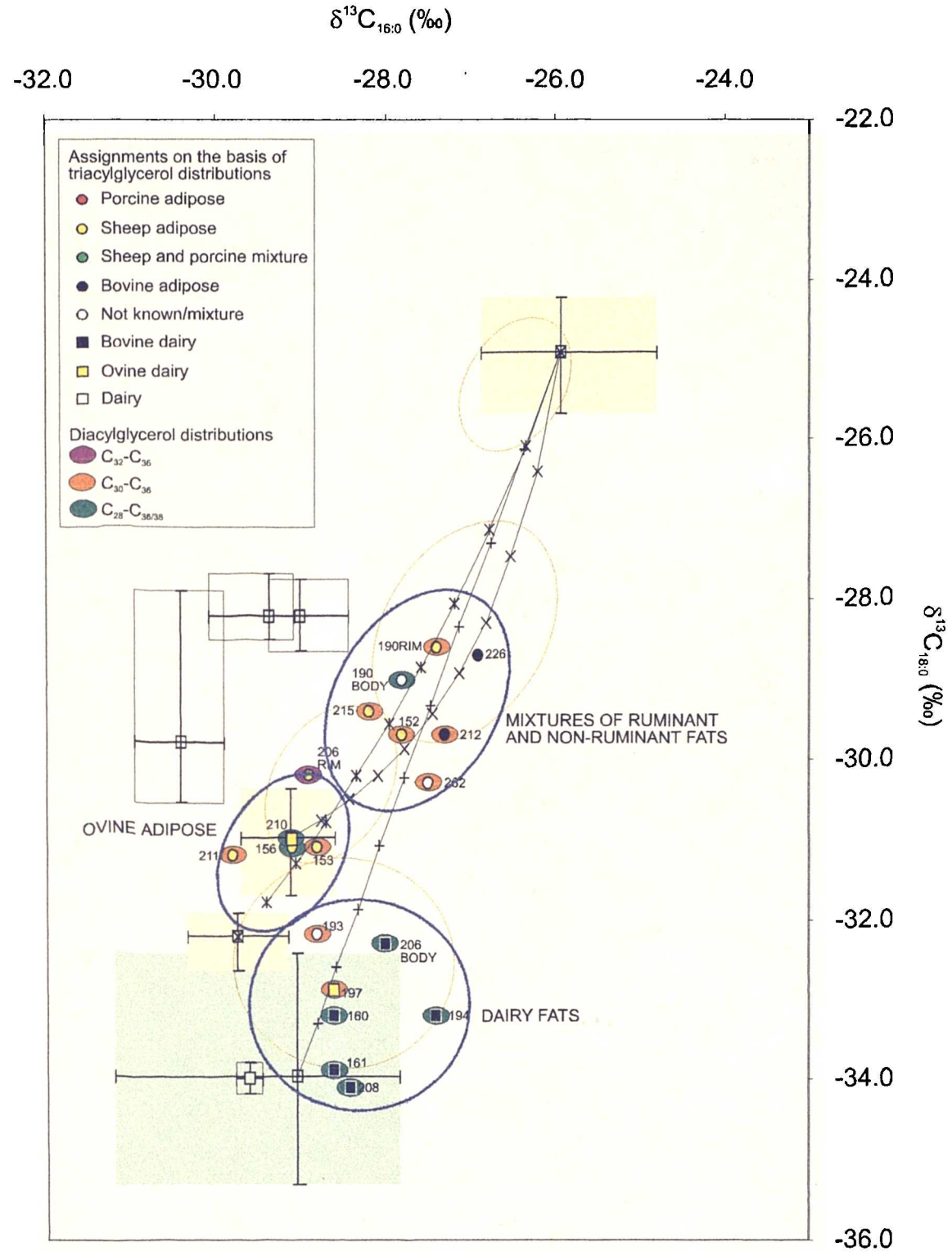


Figure 8.7 Distributions of di- and triacylglycerols in Stanwick extracts correlated with $\delta^{13}C$ values. Groups of archaeological fats distinguished by similar chemical characteristics have been indicated by the arbitrary blue rings. The approximate ranges of $\delta^{13}C$ data for groups of fats identified in the West Cotton assemblage are shown for comparison (orange rings).

ruminant fats. The porcine fats at West cotton are less enriched than the modern reference pigs; no data is available from porcine fats from Stanwick for comparison.

8.2.2 Sites with an unusually strong bias in the faunal record

8.2.2.1 Wicken Bonhunt (Romano-British/Middle Saxon)

In contrast to the data from West Cotton and Stanwick, the isotopic and distributional characteristics of the lipid residues from the late Saxon site of Wicken Bonhunt indicate that only a very limited range of animal products were available, since only ovine and porcine adipose fats have been identified. The data are similar for all of the extracts, with sample nos. WKB8, 12, 13 and 15 showing characteristics of both ruminant and non-ruminant adipose fats (Figs. 8.8 and 8.9). The remnant fats comprise $C_{16:0}$:$C_{18:0}$ fatty acid ratios similar to ovine fats, $C_{18:1}$ positional isomer distributions similar to non-ruminant fats or mixtures of fats and stable carbon isotope data correlating with the theoretical values for mixtures of ruminant and non-ruminant adipose fats. The extract from WKB16 is most closely representative of a non-ruminant fat, comprising relatively enriched $\delta^{13}C$ values, a high $C_{16:0}$:$C_{18:0}$ ratio, a relatively narrow triacylglycerol distribution, and a range of $C_{18:1}$ positional isomers indicating a contribution from a ruminant fat. This latter criterion could be affected by the diet of the animal, since *trans*-configured unsaturated components are known to be absorbed from dietary fat by non-ruminants. The ovine fats are probably all derived from sheep, as indicated from horn core evidence from the site. Pigs were believed to have been a major source of meat and fat at this site and appear to be well represented, mixed with ruminant fats in the pottery vessels. However, residues of sheep fat and/or meat are more frequent amongst the vessels analysed which is somewhat surprising since less than 20% of the faunal remains were derived from sheep. It is possible that the relatively small number of vessels analysed are not representative of the whole assemblage, but it may be that porcine meats were processed in ways other than by cooking in pots, e.g. by spit roasting, or were preserved by curing. Indeed, if this site was a distribution centre supplying pork meat to the hinterlands and nearly markets, it is possible that the consumption at the site itself was low and not reflective of the proportion of pig bones recovered. Ageing data based on mandibles and epiphyseal fusion of the sheep long bones from Wicken Bonhunt indicate that sheep were kept for some combination of wool and mutton since a sizeable number of animals were killed between 2 and 4 years of age, but

that many sheep survived to advanced ages. The interpretation of the faunal data supports the evidence from the pottery residues that sheep were exploited for their meat, rather than for milk production since no dairy residues have been identified. Cattle were the second most common domestic species at this site during the Middle Saxon period after pigs, and age profiles based upon both dental eruption and wear (Grant, 1982) and epiphyseal fusion of the long bones (Silver, 1969) clearly showed that the majority of cattle recovered from Wicken Bonhunt were mature to elderly animals. This would indicate that cattle were kept for purposes other than meat and milk production and it has been suggested that many of these animals represent mature plough oxen (Crabtree, 1995). The lipid residue data also support this observation, since no dairy or adipose fats from cattle have been identified in association with the pottery assemblage. Since there was believed to be such strong emphasis on trading between this and other sites, it is possible that in return for meat, e.g. from pigs, they were importing dairy products ready made, e.g. butter and cheese, rather than processing it themselves in pottery vessels.

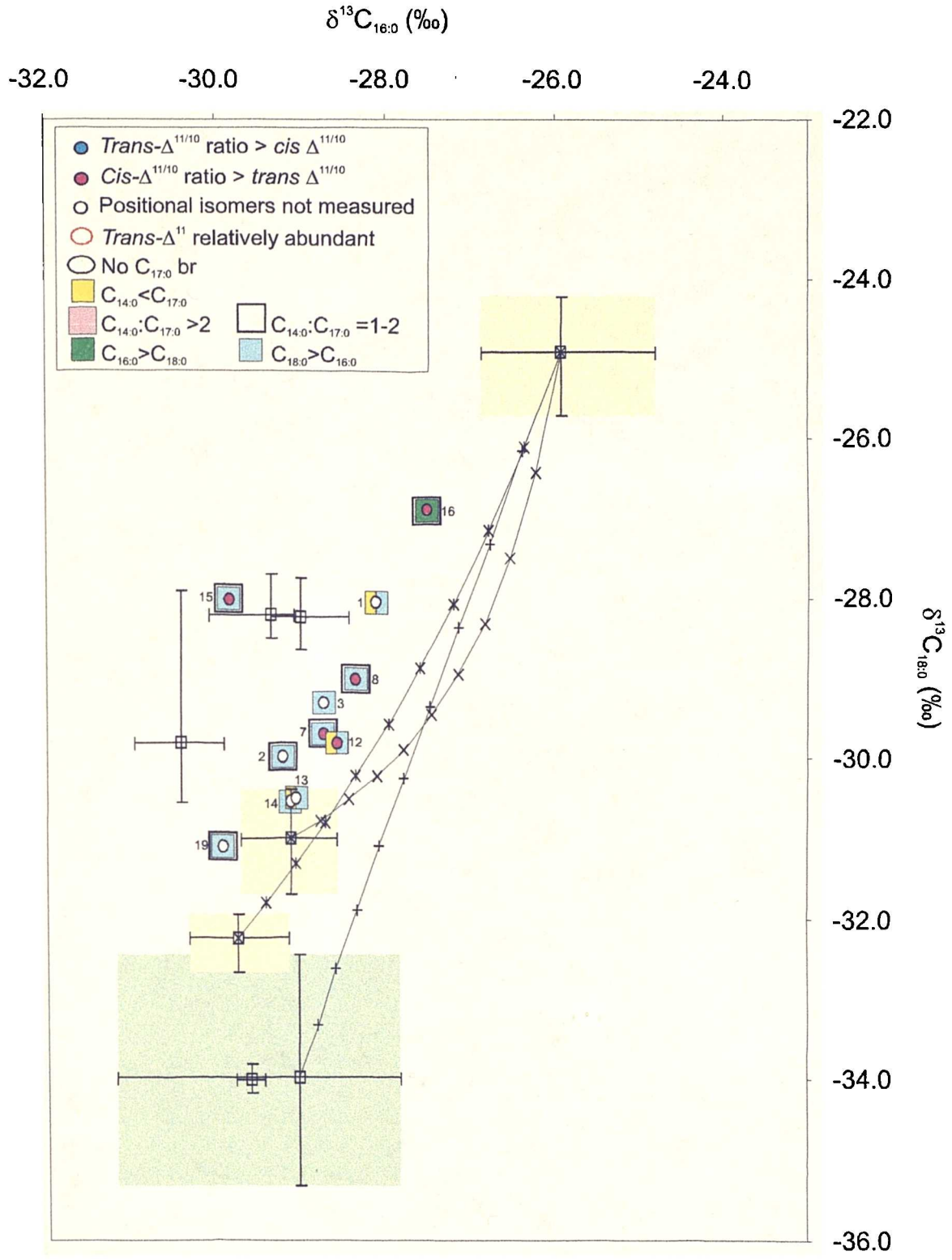


Figure 8.8 Distributions of fatty acids, including saturated $C_{14:0}$, $C_{16:0}$, $C_{17:0}$ and $C_{18:0}$ components and $C_{18:1}$ positional and geometric isomers correlated with $\delta^{13}C$ values in remnant fats from the Wicken Bonhunt assemblage.

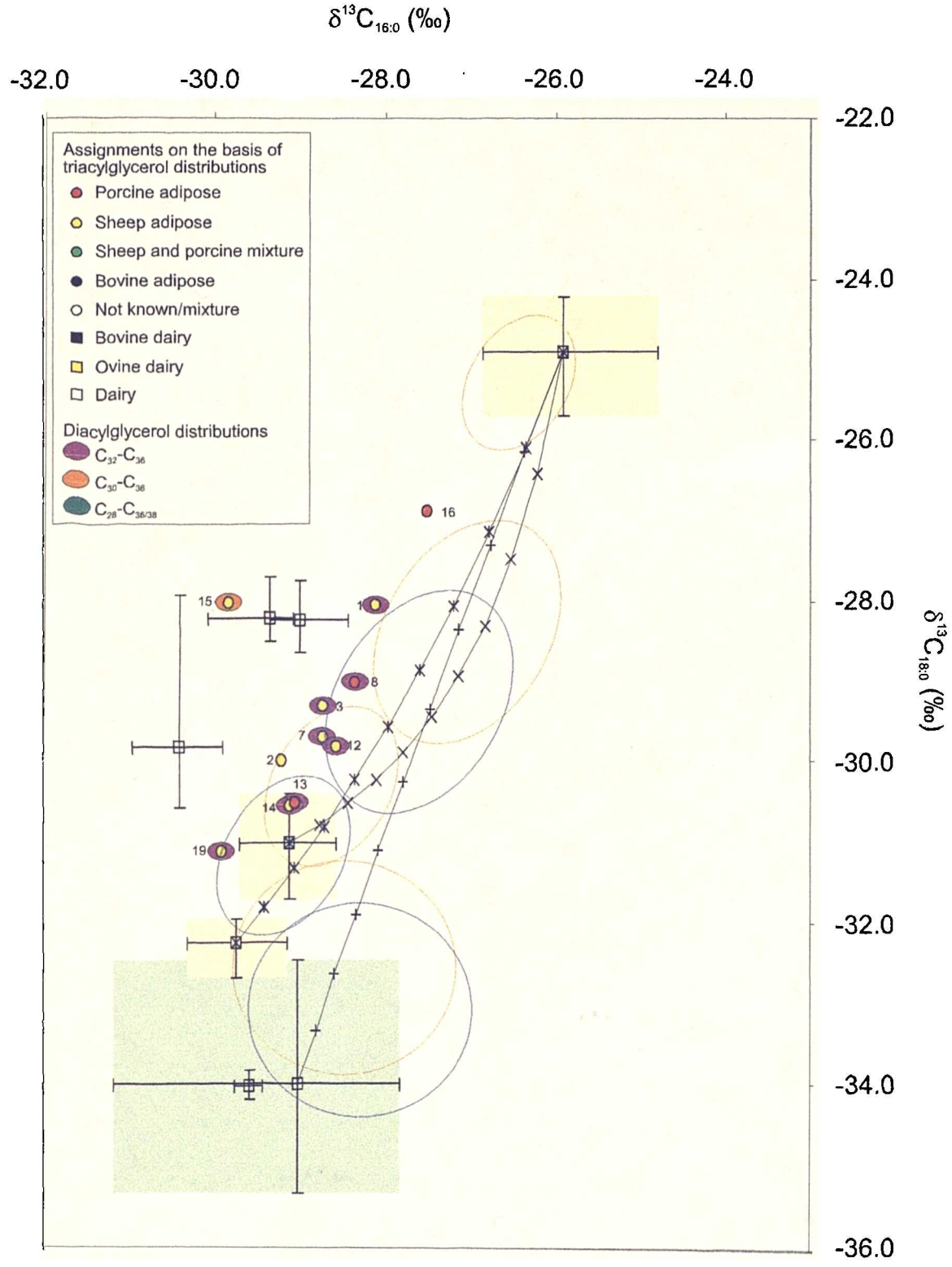


Figure 8.9 Distributions of di- and triacylglycerols in Wicken Bonhunt extracts correlated with $\delta^{13}C$ values. The approximate ranges of $\delta^{13}C$ data for groups of fats identified in the extracts from West Cotton (orange rings) and Stanwick (blue rings) are shown for comparison.

8.2.2.2 Botai, Kazakhstan (early Neolithic)

All of the remnant fats from the Botai assemblage exhibit similar characteristics, including: i) a lack of branched-chain $C_{17:0}$; ii) a higher abundance of the $C_{16:0}$ than $C_{18:0}$ fatty acid; iii) a relatively high abundance of the $C_{14:0}$ component, and iv) $\delta^{13}C$ values clustered on the mixing curve, intermediate between the data for the ruminant and non-ruminant reference fats. The combination of criteria strongly indicate a common source for all of the remnant fats. On the basis of comparison with data from the Siberian horse fats, these archaeological residues all appear to represent degraded horse fat. The isotopic data from the modern reference horses raised in the UK are markedly different, indicating the magnitude of the effect that a difference in the isotopic composition of the diet can have on the fat composition of a non-ruminant animal.

8.2.3 Prehistoric archaeological sites

8.2.3.1 Yarnton Cresswell field (early-middle Iron Age)

Eleven of the 19 extracts from the Yarnton Cresswell field assemblage exhibited the isotopic and distributional characteristics of ruminant dairy fats, the majority believed to be derived from bovine adipose, with two from an ovine source (Figs. 8.11 and 8.12). The identifications of bovine dairy fats have been made on the basis of the intact triacylglycerol distributions and relatively high abundances of the $C_{14:0}$ and $C_{16:0}$ components. Indeed, a high proportion of the extracts generally contain a relatively high $C_{14:0}$:$C_{17:0}$ ratio, indicative of the relatively good preservation afforded to the free fatty acids in the extracts. The $\delta^{13}C$ values of the dairy fats were more variable than at Stanwick or West Cotton, but similarly plotted with a mean value more enriched (ca. 1‰) than the mean of the modern reference data. Extracts from the base, body and rim sherds of a slack-shouldered jar of fabric type GSA4 and the rim sherd of a tripartite jar have been identified as dairy fats. A number of remnant fats exhibit more enriched $\delta^{13}C$ values than the ruminant adipose and are characterised by narrow distributions of diacylglycerols seen in porcine fats but with distributions of intact triacylglycerols similar to ovine adipose fats, indicating that they represent mixtures of fats. Samples 112, 113 and 144 appear to be distinguishable from samples 148, 149, 119, 101 and 126 by their low $C_{14:0}$:$C_{17:0}$ ratio, although this may just be a reflection of the extent of decay of the shorter-chain fatty acid components. Samples 119 and 149 appear to derive from the same source, exhibiting $\delta^{13}C$ values which correspond to

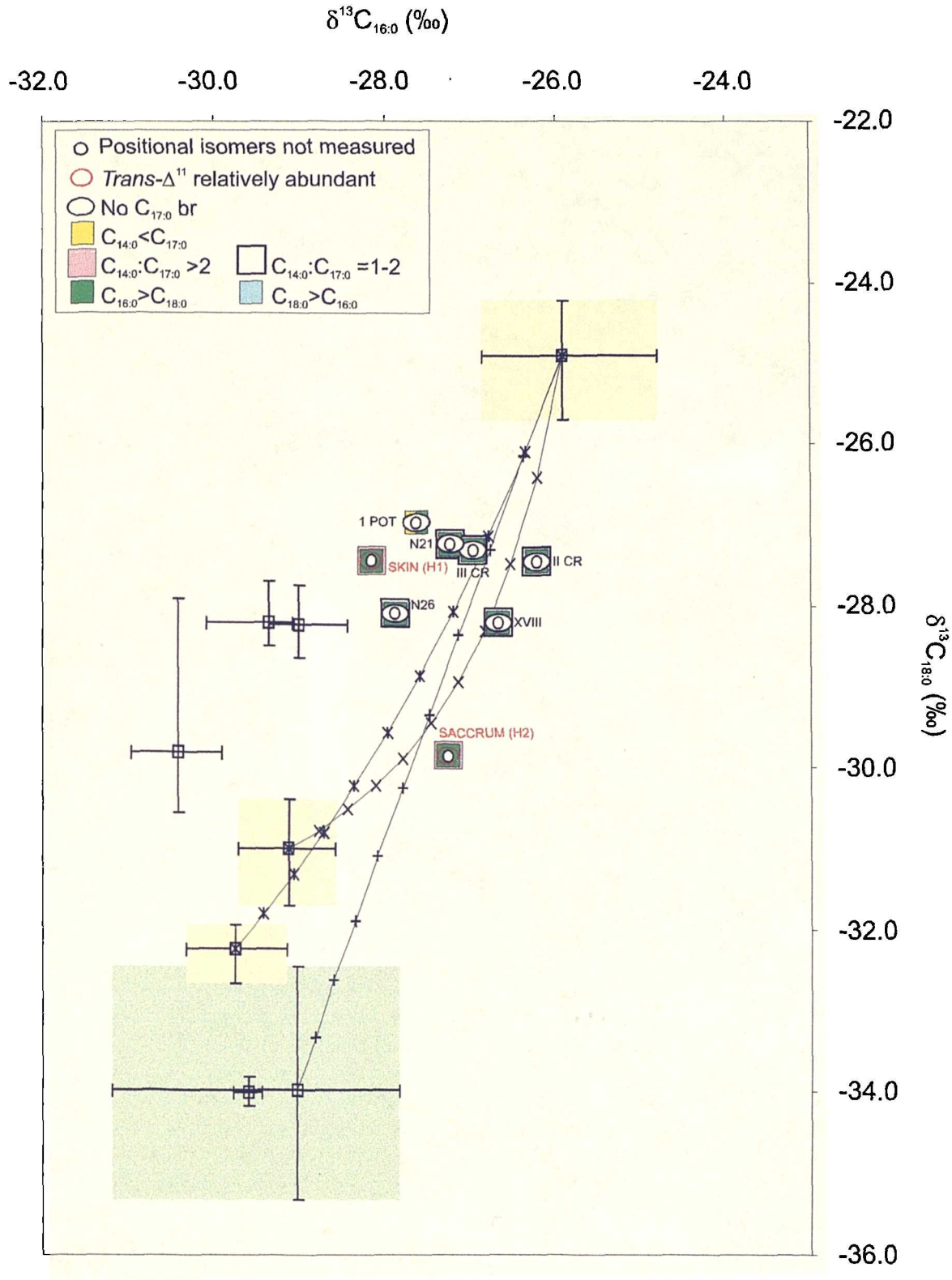


Figure 8.10 Distributional and isotopic data obtained for the extracts from the Botai pot sherds compared with data for the Siberian horse fats.

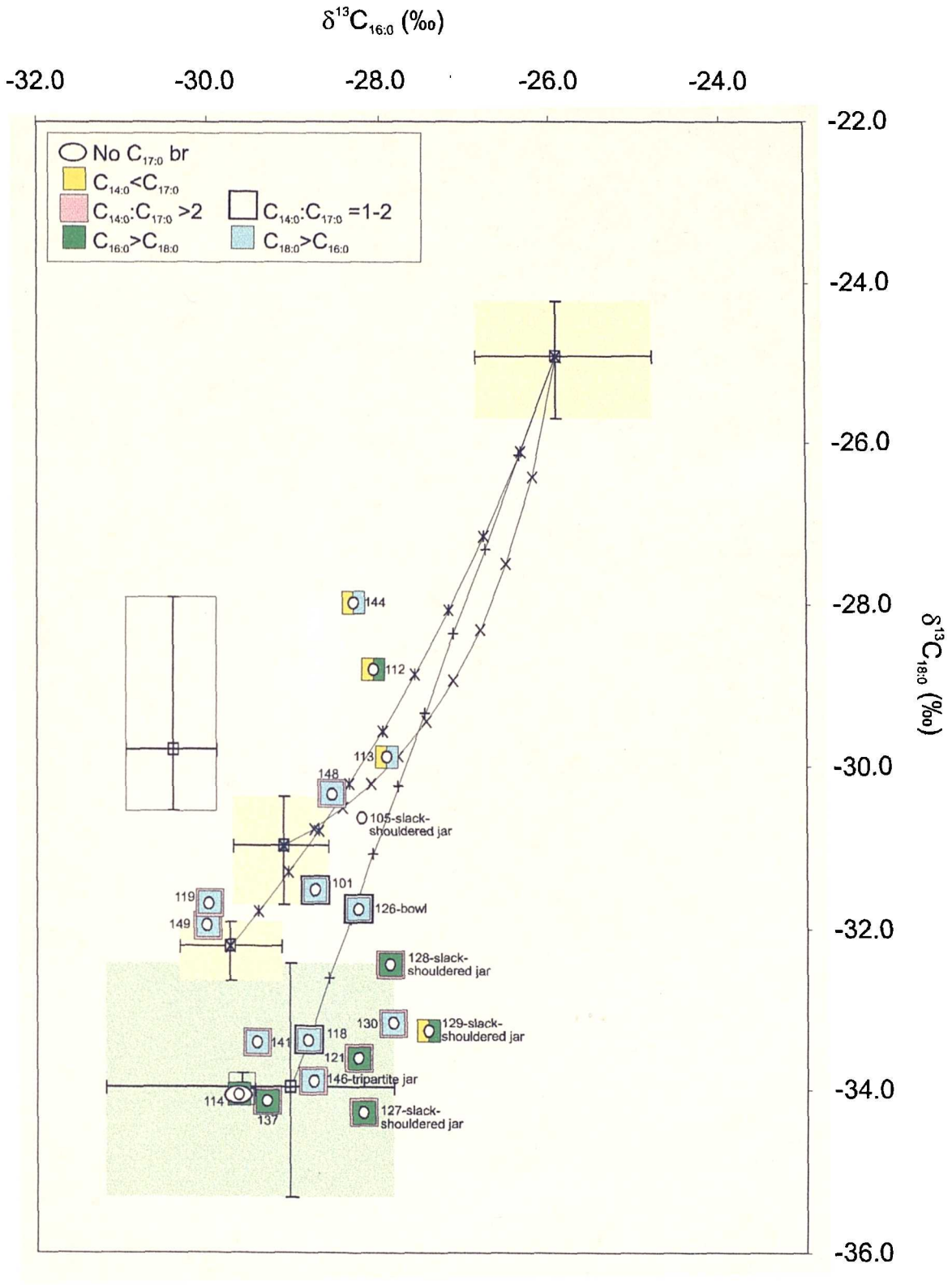


Figure 8.11 Distributions of fatty acids, including saturated $C_{14:0}$, $C_{16:0}$, $C_{17:0}$ and $C_{18:0}$ components correlated with $\delta^{13}C$ values in remnant fats from the Yarnton Cresswell field assemblage.

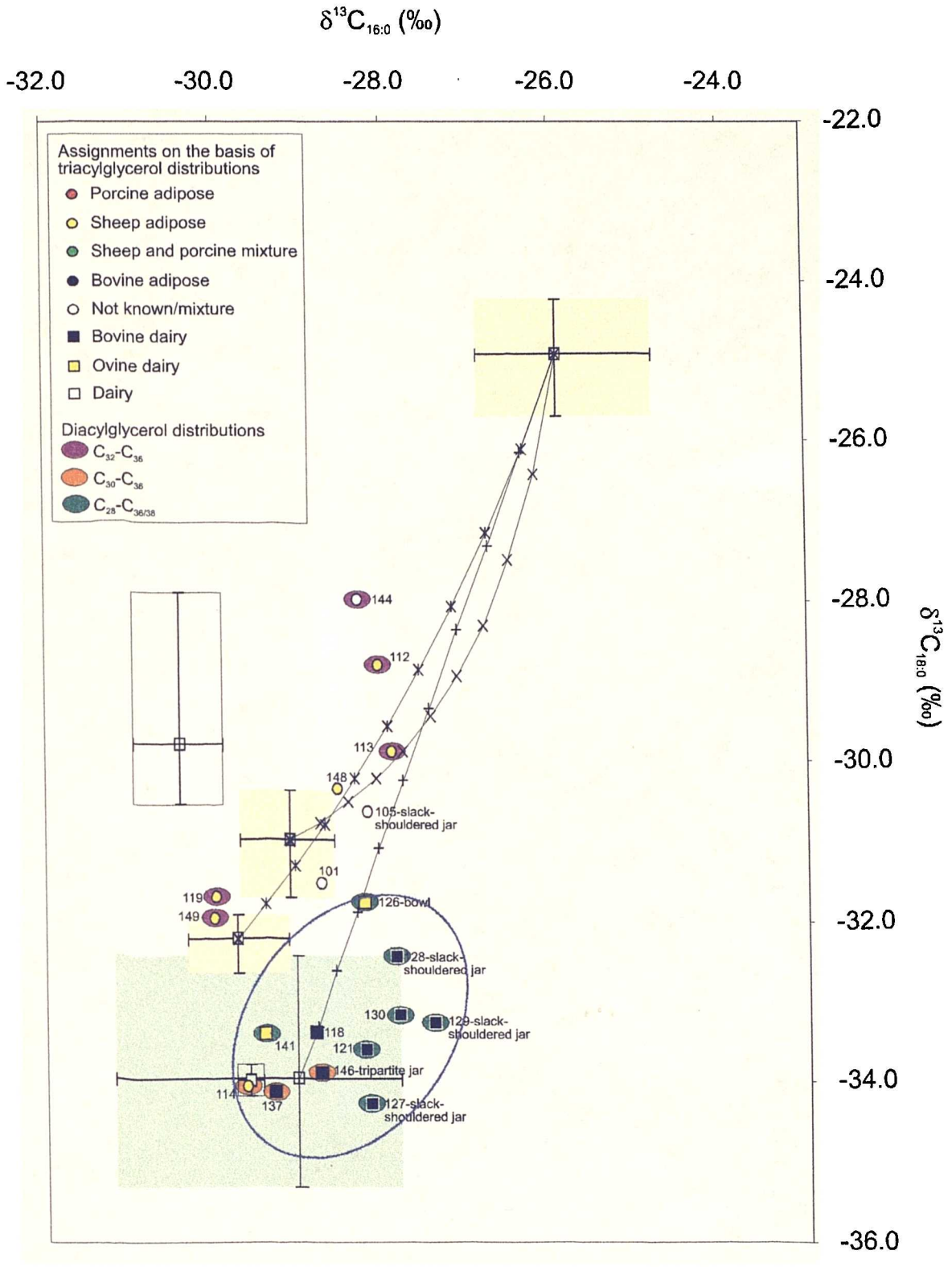


Figure 8.12 Distributions of di- and triacylglycerols in Yarnton Cresswell field extracts correlated with $\delta^{13}C$ values. The range (blue ringed area) encompassing the $\delta^{13}C$ data points for remnant fats identified as having a dairy origin is shown.

the ruminant adipose fats, a narrow range of diacylglycerols as seen in porcine fats, and a similar distribution of triacylglycerols and free fatty acids as seen in ovine adipose fats. A large proportion of the fats appear to derive from an ovine origin due to the higher abundance of the $C_{18:0}$ than the $C_{16:0}$ fatty acid. In these extracts the relatively high $C_{14:0}$:$C_{17:0}$ ratio is an indication that the $C_{16:0}$:$C_{18:0}$ ratio has survived intact. None of the extracts exhibit the relatively enriched $\delta^{13}C$ values characteristic of porcine fats, and as at the archaeological sites already considered, none of the remnant fats are identifiable as bovine adipose. If this is a true representation, then it appears that bovines were not kept for their meat, but rather for milk production and/or as traction animals. Ovines appear to have been kept for both their meat and milk.

8.2.3.2 Yarnton flood plain (Neolithic-Bronze Age)

Lipid residues from both Yarnton flood plain and Yarnton Cresswell field are comparable, with both assemblages comprising remnant animal fat residues in a similar state of preservation. Indeed, the preservation of the remnant fats from these early sites is remarkable. The isotopic data shown in Figures 8.13 and 8.14 indicate that the majority of the extracts derive from ruminant animals. Seven remnant fats have been identified as dairy fats on the basis of di- and triacylglycerol distributions, relatively high abundances of the $C_{14:0}$ fatty acid and more highly depleted $\delta^{13}C$ values, particularly for the $C_{18:0}$ fatty acid. These include sample nos. 30, 31, 41, 23, 5 and 4. The presence of dairy fats from both ovine and bovine sources is indicated by the intact triacylglycerol distributions. Sample 50 is probably also derived from an ovine dairy fat as indicated by the $\delta^{13}C$ values. The distributions of di- and triacylglycerols and the $\delta^{13}C$ values have enabled the identification of a porcine fat in sample 38. Anomalies include sample 1 which corresponds to the stable isotope composition of a ruminant adipose fat, however, exhibits the distributional characteristics of a dairy fat. Sample 43 corresponds to a mixture of porcine and ruminant adipose fats, although the $\delta^{13}C$ data indicate that the extract is composed of a higher proportion of fat from a ruminant source. Thus the Yarnton flood plain extracts comprised 9 dairy fats, 1 porcine fat and 1 ruminant adipose (possibly mixed with a minor proportion of non-ruminant fat), indicating the use of vessels largely for the processing and/or storage of dairy fats and the exploitation of both ovine and bovine animals for their milk. Both ovine and porcine animals appear to have been exploited for their meat, however, the

infrequent occurrence of adipose fat residues in the vessels suggests either meat was rarely consumed, in favour of keeping animals for dairying, or pottery vessels were not used to store or process animal meat/fats. Porcine animals are not well represented at either of the Yarnton sites, but are definitely present at the earlier flood plain site. During both periods of occupation dairy fats from both cattle and sheep (or goats) were commonly processed or collected in pottery vessels, with the slightly more frequent use of vessels in processing ruminant animal products at the later Cresswell field site.Correlation of the data with vessel type indicates that dairy fats were processed in mid-Neolithic Peterborough ware, Fengate ware and Mortlake ware and two Early Bronze Age beakers, whereas the one example of porcine adipose fat identified was recovered from a late Neolithic Grooved ware vessel.

8.2.3.3 Eton Lake End Road (late Neolithic-Early Bronze Age)

The extent of decay in the Lake End Road extracts is more significant than in remnant fats retrieved from the Yarnton potsherds, evidenced by the low $C_{14:0}$:$C_{17:0}$ ratio in the extracts characterised as dairy fats (Fig. 8.15). Ten of the 12 extracts have been identified as dairy fats on account of their highly depleted $\delta^{13}C$ values and broad triacylglycerol distributions (Fig. 8.16). The majority, if not all, of the dairy fats derive from a bovine origin, indicated by the relative abundances of the intact triacylglycerols. Several extracts, including sample nos. 1, 2-8, 2-rim and 8-2166, exhibit the narrow diacylglycerol distributions more closely representative of adipose fats, however, they show relatively depleted $\delta^{13}C$ values and broad ranges of triacylglycerols consistent with a ruminant dairy origin. All four sherds from different parts of the profile of NRA 2 have been identified as degraded dairy fats, probably from a bovine origin due to the relative abundance of the C_{50} triacylglycerol component. None of the remnant fats correspond exactly to the $\delta^{13}C$ values for the modern reference porcine fats, however, samples 4 and 8-2164 and 10 contain distributions of di- and/or triacylglycerols markedly characteristic of porcine fats. Since the distributional characteristics of degraded porcine fats are so distinctive, it is very unlikely that ruminant animal fats could resemble non-ruminant fats regardless of the extent of decay. Although the $\delta^{13}C$ values in samples 4 and 8-2164 are more depleted than would be expected for non-ruminant fats, they undoubtedly comprise a proportion of fat from a non-ruminant origin, perhaps from animals raised on isotopically light diets or mixed with fats from another source.

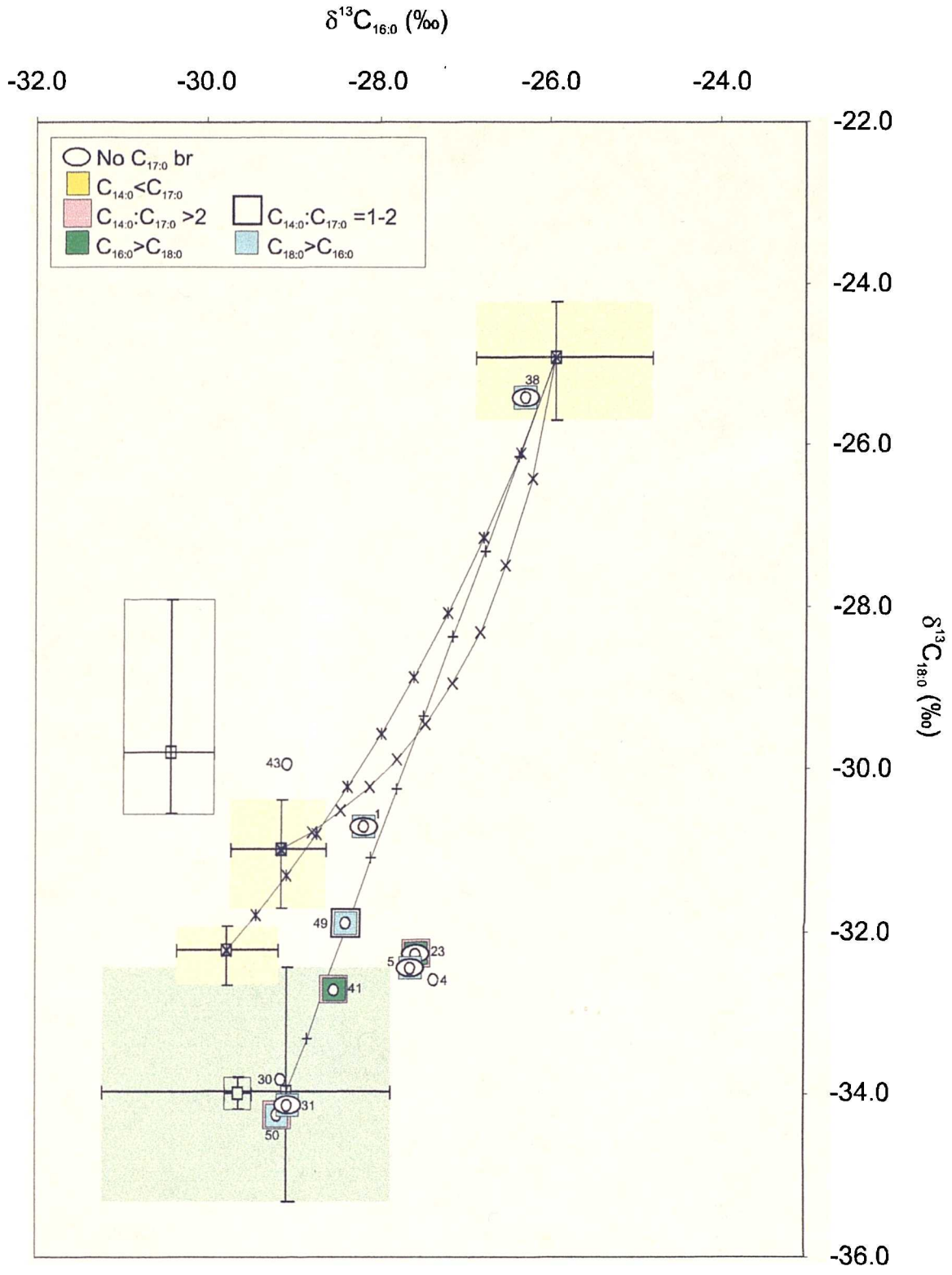


Figure 8.13 Distributions of fatty acids, including saturated $C_{14:0}$, $C_{16:0}$, $C_{17:0}$ and $C_{18:0}$ components correlated with $\delta^{13}C$ values in remnant fats from the Yarnton flood plain assemblage.

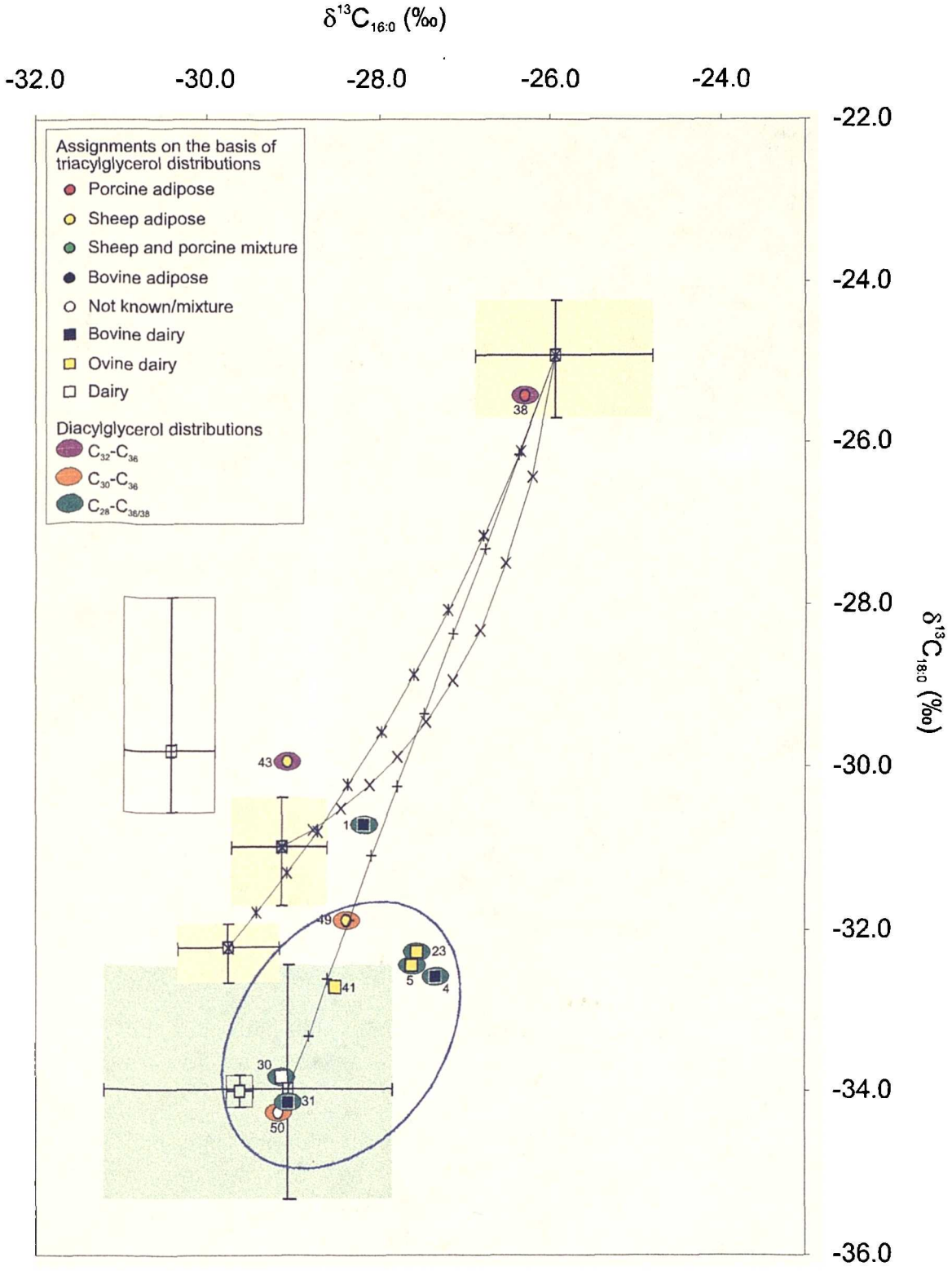


Figure 8.14 Distributions of di- and triacylglycerols in Yarnton flood plain extracts correlated with $\delta^{13}C$ values. The range (blue ringed area) encompassing the $\delta^{13}C$ data points for remnant fats identified as having a dairy origin is shown for comparison with the Yarnton Cresswell field and Eton sites.

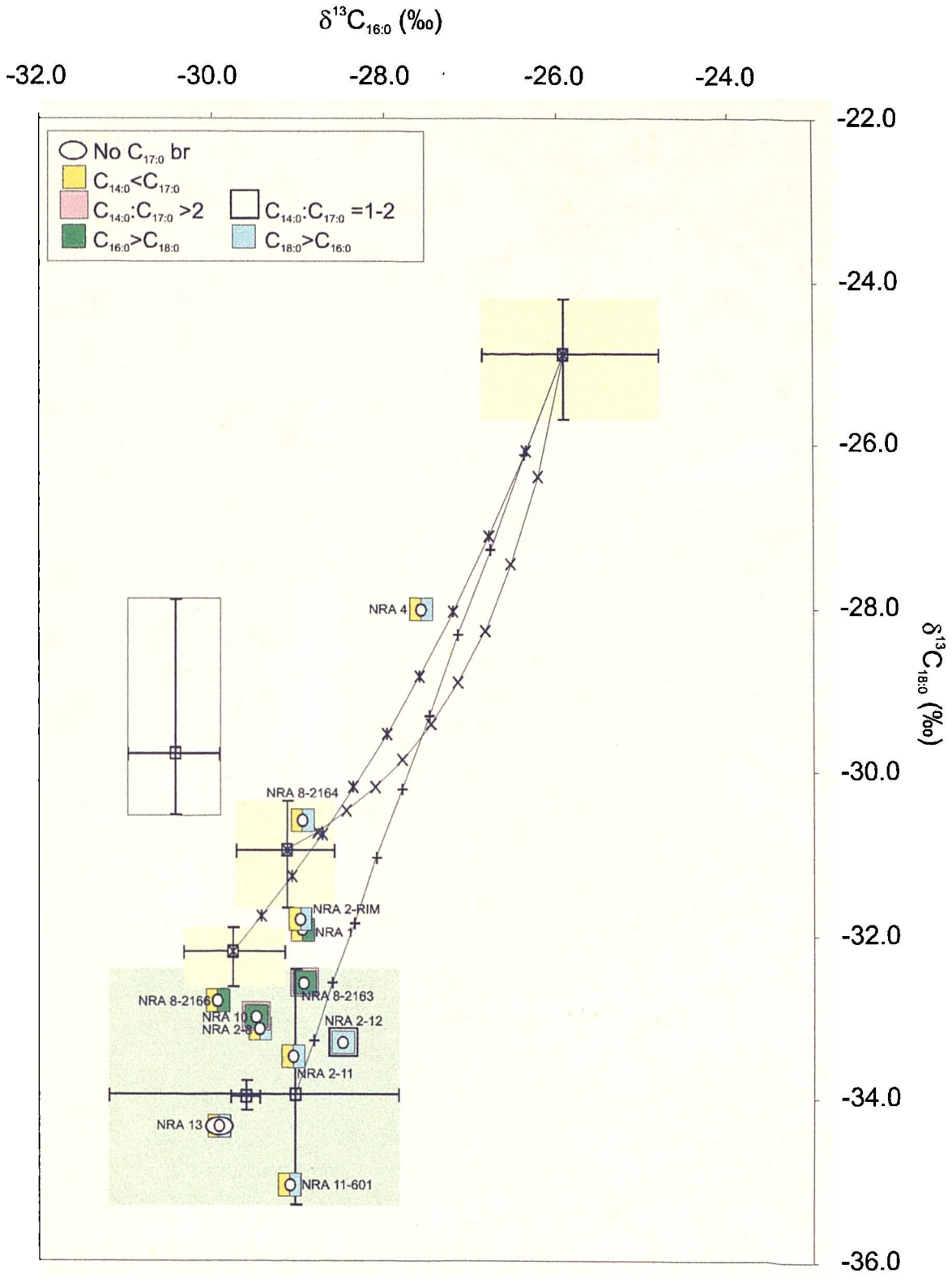


Figure 8.15 Distributions of saturated $C_{14:0}$, $C_{16:0}$, $C_{17:0}$, $C_{18:0}$ correlated with $\delta^{13}C$ values in remnant fats from the Eton Lake End Road assemblage.

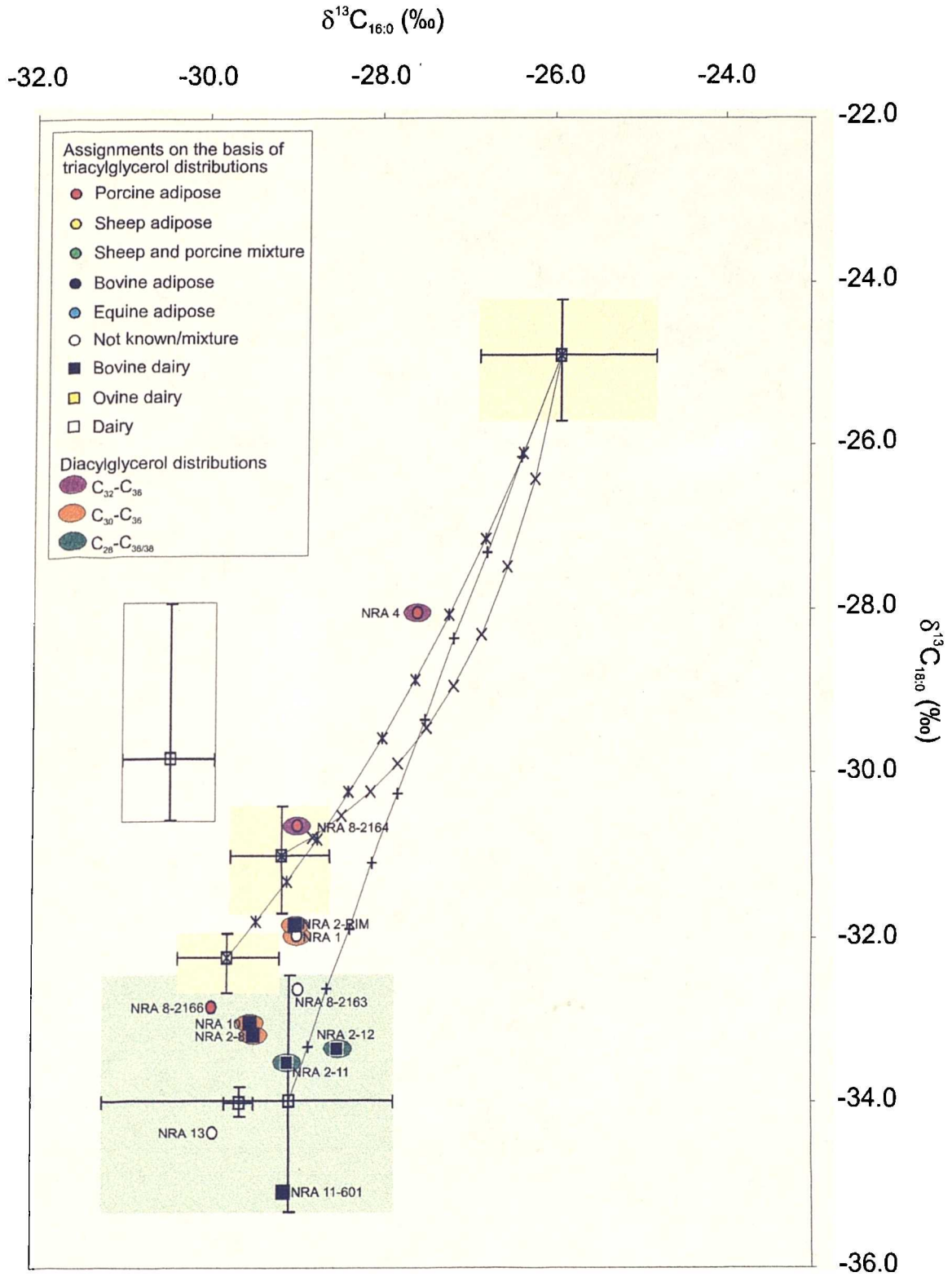


Figure 8.16 Distributions of di- and triacylglycerols in Eton Lake End Road extracts correlated with $\delta^{13}C$ values.

8.2.3.4 Eton Rowing Lake (early Neolithic)

The stable carbon isotope data indicate that the majority of the remnant fats from Eton Rowing Lake are of a ruminant dairy origin, although other chemical criteria do not wholly support these assignments (Figs. 8.17 and 8.18). The depleted isotopic data and broad triacylglycerol distributions are characteristic of a dairy origin, however, the diacylglycerol distributions in certain extracts only range between C_{30} to C_{36} which are more characteristic of ruminant adipose fats. Many of the remnant fats exhibiting relatively depleted $\delta^{13}C$ values contain relatively low $C_{14:0}$:$C_{17:0}$ ratios, indicating that decay is more extreme in these dairy residues than seen at any of the other sites. This may explain the lack of the lower carbon number diacylglycerols usually present in dairy fats and indicates that the $C_{16:0}$:$C_{18:0}$ fatty acid ratio is also unreliable. The triacylglycerol distributions in samples 1, 8 and 13a are more characteristic of ovine adipose fats than dairy fats and may have been adversely affected by decay or represent isotopically depleted fats from an ovine source or mixtures of ruminant dairy and adipose fats. Sample 22 contains relatively depleted $\delta^{13}C$ values but the distribution of triacylglycerols is very similar to that seen in the reference horse fats and the extract contains a high proportion of the $C_{14:0}$ fatty acid characteristic of both equine and dairy fats. However, the abundance of the $C_{16:0}$ fatty acid is not significantly higher than the $C_{18:0}$ fatty acid for this remnant fat to have derived from an equine source, and since the isotopes are also incomparable with reference horse fats it probably represents a bovine adipose or dairy fat. None of the extracts are characteristic of porcine fats or resemble mixtures of ruminant and non-ruminant fats. Thus, based on the chemical analyses of residues from Eton Rowing Lake the vessel function appears to have been predominantly for the collection and/or processing of dairy products rather than for the processing of meat or body fats. The data correspond with those from the later Eton site, where the degraded animal fats exhibit distributional characteristics similar to adipose fats but show significantly more depleted $\delta^{13}C$ values than would be expected. Despite the isotopic anomalies noted amongst these samples, there is evidence at the Rowing Lake site for the processing of both ovine and bovine dairy fats but not non-ruminant fats, whilst at the later Lake End Road site we have identified remnant fats exhibiting characteristics of non-ruminant adipose fats and bovine dairy fats, but little evidence for the exploitation of ovine species.

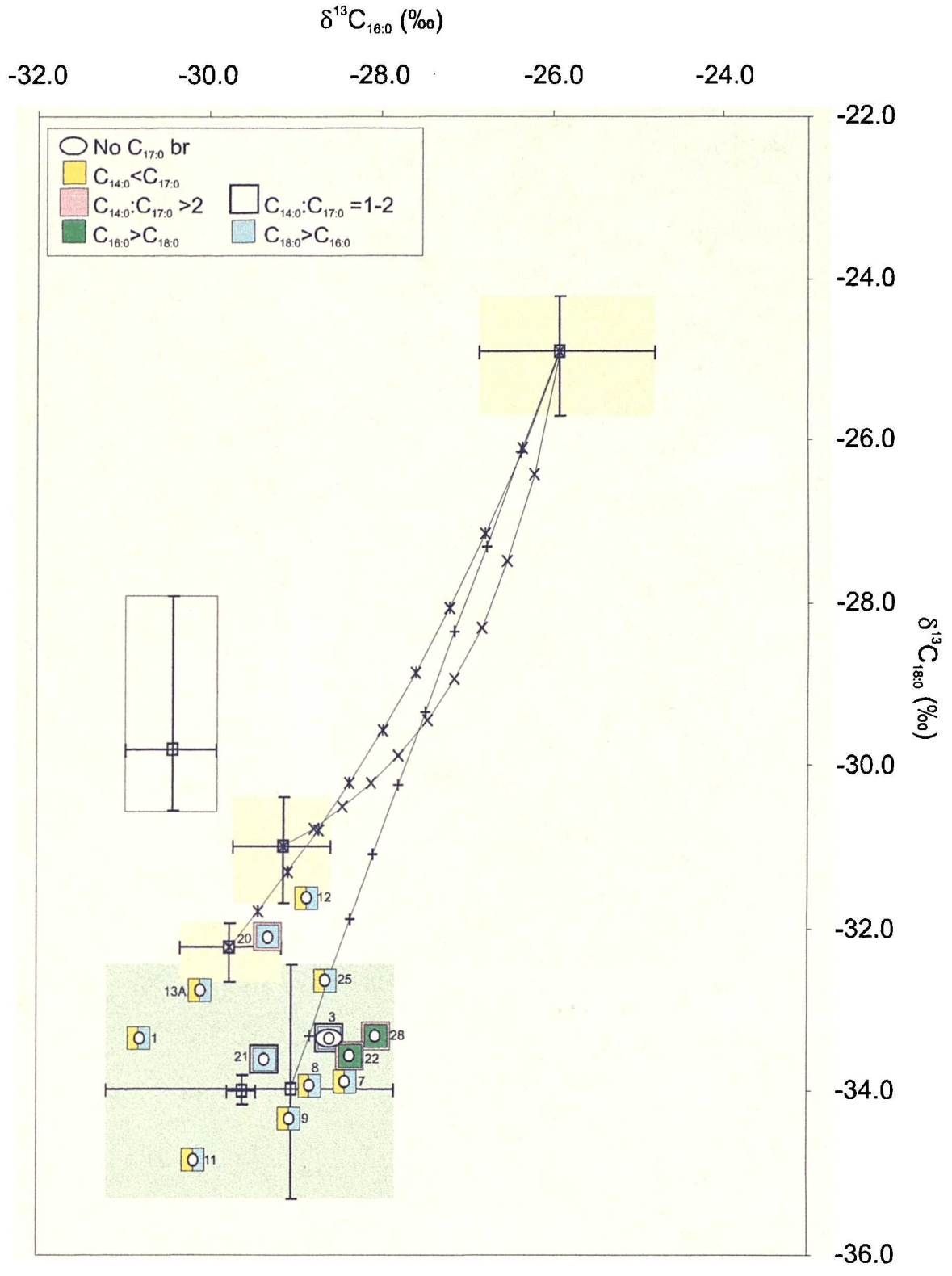


Figure 8.17 Distributions of fatty acids, including saturated $C_{14:0}$, $C_{16:0}$, $C_{17:0}$ and $C_{18:0}$ components correlated with $\delta^{13}C$ values in remnant fats from the Eton Rowing Lake assemblage.

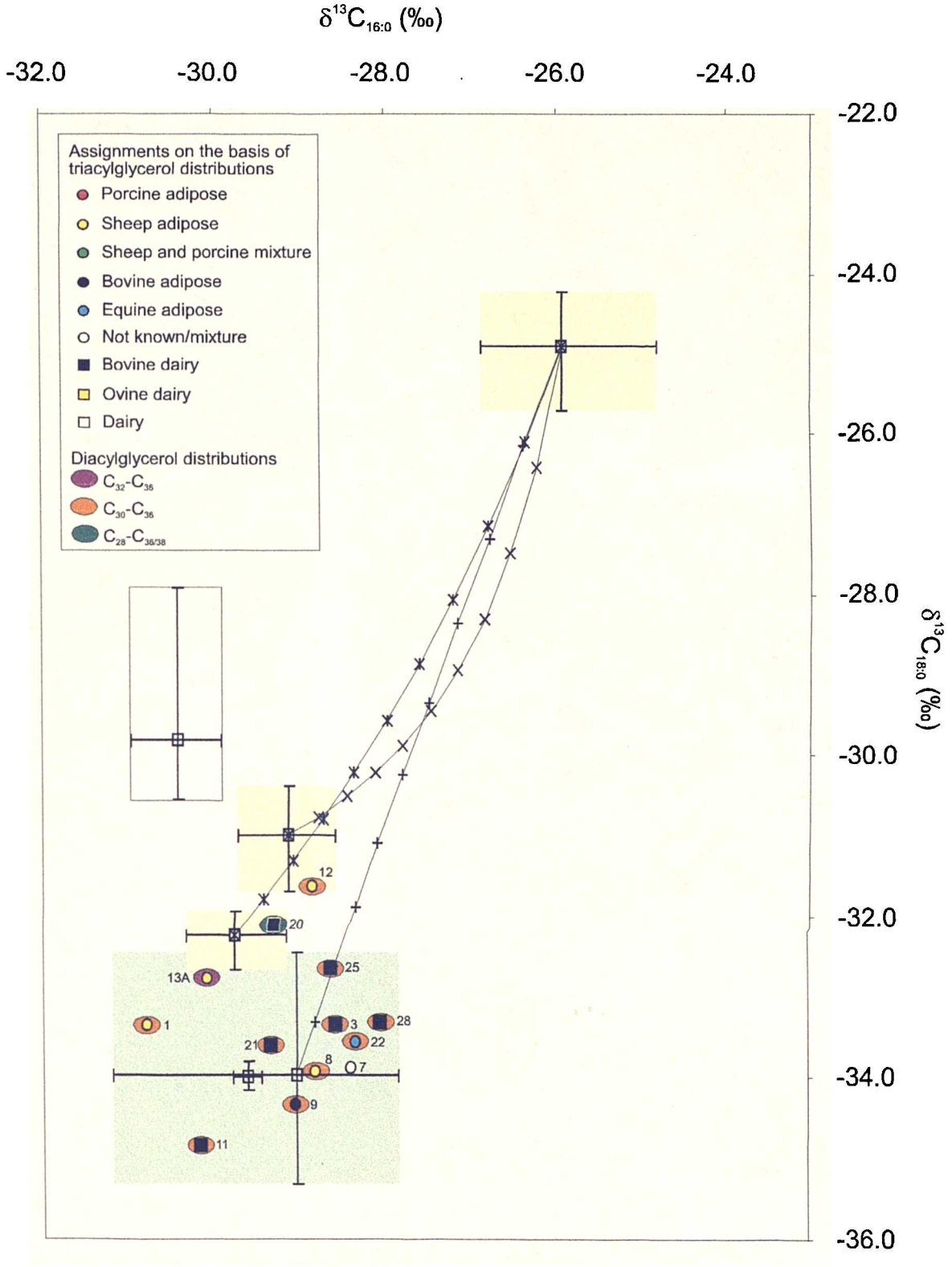


Figure 8.18 Distributions of di- and triacylglycerols in Eton Rowing Lake extracts correlated with $\delta^{13}C$ values.

8.2.3.5 Upper Ninepence (early-late Neolithic)

Some of the earliest pottery studied as part of this project has come from the Neolithic site of Upper Ninepence at Walton, mid Wales, where excavations revealed two phases of occupation associated with two different ceramic traditions, namely Grooved ware (2500 BC) and Peterborough ware (3000 BC). The Grooved ware and Peterborough ware pits seem to have a mutually exclusive distribution on the site. Screening of the sherds for lipid residues has revealed the presence of remnant fats in a remarkably well-preserved state considering the age of the finds. This small assemblage of sherds yielded animal fats comprising abundant intact acyl lipids, while no faunal remains survived, thus illustrating the importance of the clay matrix in protecting lipid residues from chemical and biological decay processes.

The $\delta^{13}C$ values clearly show a distinction between the remnant fats from the absorbed residues from the Peterborough ware and the Grooved ware, supported by differences in the diacylglycerol and intact triacylglycerol distributions in the extracts. The data indicate that the Peterborough ware extracts derive from ruminant fats whilst the absorbed residues from the Grooved ware derive from non-ruminant, e.g. porcine fats. The non-ruminant fats are distinguished by a distinctly narrow range of both the di- and triacylglycerols and significantly less depleted $\delta^{13}C$ values than the other remnant fats (Fig. 8.19). The carbonised residues from the Grooved ware have been identified as deriving from ruminant dairy fats. The triacylglycerol distributions in the ruminant fats from the different vessel types are distinguished by slightly lower abundances of the C_{42}, C_{44} and C_{46} triacylglycerols in the remnant fats from the Peterborough ware. The relative abundances (% total saturated components) of $C_{14:0}$ straight-chain and $C_{17:0}$ branched-chain fatty acids in the archaeological extracts has also been considered. The data show higher proportions of both these components in the ruminant fats from the Grooved ware than in the non-ruminant fats from different vessels in the same assemblage and the remnant ruminant fats in the Peterborough ware. The data support different origins for the fats from the absorbed and carbonised residues from the Grooved ware assemblage. Furthermore, the remnant ruminant fats in the carbonised residues from the Grooved ware comprise a higher abundance of the $C_{14:0}$ fatty acids than the ruminant fats from the Peterborough ware, indicating that the latter may derive from a different source. The mean ratios of abundance

of the $C_{14:0}$ and $C_{17:0}$ branched-chain components are 1.4, 0.5 and 2.2, for the absorbed residues in the Peterborough ware and the absorbed and carbonised residues in the Grooved ware, respectively. In the modern reference fats this ratio is lower in sheep milk than in cow's milk and lower still in ruminant adipose fats. Thus, it is possible that the fats in the Peterborough ware derive from ruminant adipose or ovine milk. The presence of C_{42}-C_{46} triacylglycerols in the Peterborough ware extracts supports the latter, however, these data are somewhat conflicting since the relative abundance of the C_{54} triacylglycerol would be expected to be somewhat higher in an ovine dairy fat. The fatty acid components in sample P5 are poorly preserved and the abundances of both $C_{14:0}$ and $C_{17:0}$ branched-chain fatty acids are present in low abundance. It is possible that the differences we are seeing between the ruminant fats from the two assemblages are as a result of preferential decay of the lower carbon-number components from the Peterborough ware extracts and that in fact the ruminant fats in the Grooved ware and the Peterborough ware derive from the same source.

Mid-chain ketones (C_{31}, C_{33} and C_{35}) have been identified in two of the Peterborough ware vessels, indicating that the ruminant fats were being processed at very high temperatures in these vessels. Experimental work would be able to determine whether on heating dairy fats reach temperatures high enough to allow the formation of mid-chain ketones before they burn. It would seem more likely that the high temperatures that lard reaches, e.g. during frying, would be more conducive to the formation of mid-chain ketones. The thick carbonised residues from the Grooved ware contained abundant lipid but no mid-chain ketones. The presence of carbonised residues on the interior surface of these vessels is quite consistent with their use in the heat treatment (e.g. pasteurisation) of dairy products at reasonably high temperatures since they are particularly susceptible to burning and produce thick charred deposits.

Interestingly, the remnant non-ruminant fats from Walton are more depleted in ^{13}C by approximately 2‰ compared to the reference C_3-fed pig fats, and similarly, are more depleted than the remnant fats identified as porcine from West Cotton (the ranges of data are compared in Fig. 8.19). Figure 8.20 shows the $\delta^{13}C$ values for fatty acids in the reference pig and the archaeological fats from P66 and P68 compared with the bulk values for the diet of the reference animals. Also shown are bulk values obtained for examples of

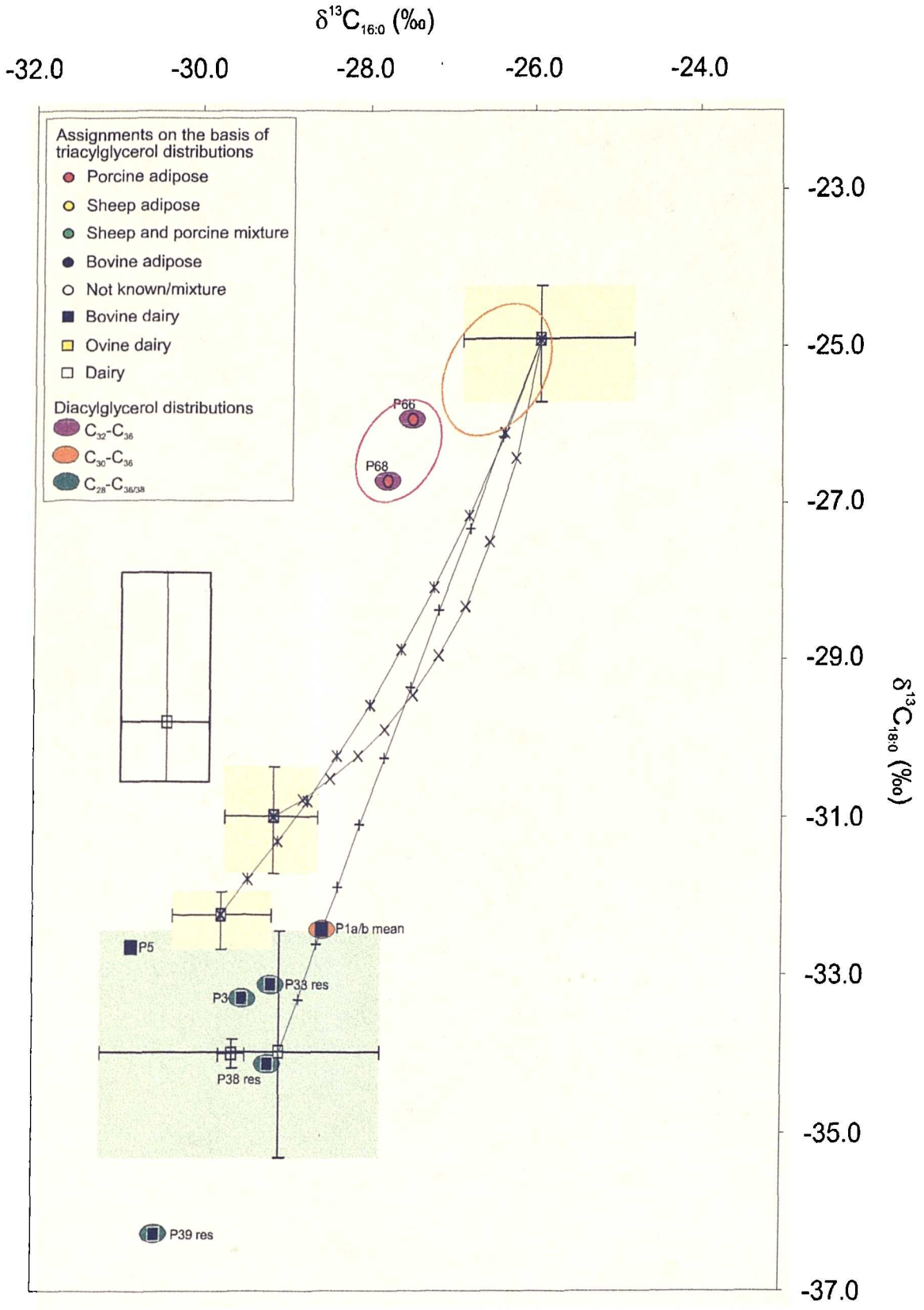


Figure 8.19 Distributions of di- and triacylglycerols in Walton extracts correlated with $\delta^{13}C$ values. The approximate range of $\delta^{13}C$ data for porcine fats identified in three extracts from West Cotton (orange ring) is shown for comparison.

foodstuffs which may have contributed to the diet of pigs in antiquity, including acorns, beech nuts and whey (Grigson, 1982; Ryder, 1983a). The $\delta^{13}C$ values of the fatty acids in the reference pig fat reflect the bulk value obtained for the diet. These values correlate very well with those reported in Stott *et al.* (1997) in studies of lipid extracts of bone from C_3-fed pigs. It is possible that the more depleted values seen for the archaeological porcine fats (compared with the reference fats) reflect a contribution in the diet from isotopically-depleted foodstuffs, such as beech nut kernels or whey which are more enriched in ^{12}C than the C_3 diet of the reference pig. It is well known that cheese-makers traditionally kept pigs to consume whey, the by-product of cheese production, and this is still the case in Romania, southern Russia and southern Poland today (Ryder 1983a, 1983b). Additionally, pigs are thought to have been allowed to root in woodland for nuts and where housed over winter they may have been fed collected nuts as an additional source of protein and carbohydrate (Grigson, 1982). Whey in the diet may also account for the fact that we have detected low abundances of branched-chain fatty acid components in the archaeological non-ruminant fats (St. John *et al.*, 1987; Busboom *et al.*, 1991) which we would not normally expect to see in porcine fats, although a possible exogenous bacterial source for these components cannot be completely ruled out.

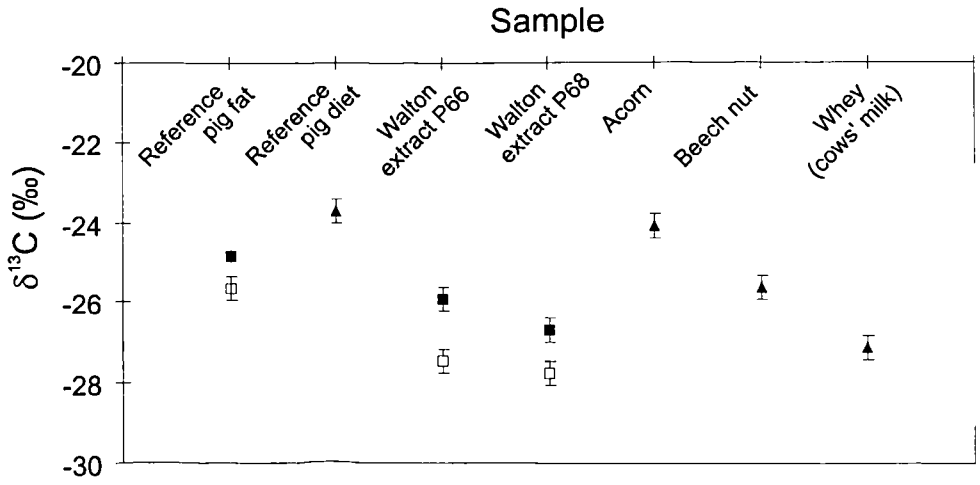


Figure 8.20 $\delta^{13}C$ values for $C_{16:0}$ and $C_{18:0}$ fatty acids in reference pig fat and archaeological non-ruminant fat from Walton compared with bulk values (▲) for diets. The $\delta^{13}C$ values for $C_{16:0}$ (□) and $C_{18:0}$ (■) fatty acids in the reference fats reflect the bulk values obtained for the diet. The values for the fatty acids in the archaeological fats may have been influenced by the contribution of ^{12}C-enriched components, e.g. nuts and whey, in the diet. Error bars indicate the instrumental error (± 0.3‰). All values are a mean of triplicate determinations.

The chemical analyses of the Neolithic pottery residues from Walton have revealed clear differences in vessel use and indicate possible changes in patterns of animal exploitation and methods of food processing and/or dietary preferences between the two phases of occupation associated with the Grooved ware and Peterborough ware. This investigation has clearly illustrated the importance of residue analysis in archaeological investigations, particularly at prehistoric sites where evidence from environmental and faunal remains is limited or absent.

8.3 Reliability and recommendations for utilising various chemical criteria

8.3.1 The use of fatty acid distributions in determining species origin

In drawing comparisons between fatty acid distributions in archaeological and modern reference fats, consideration has been given to: (i) the ratios of the major saturated fatty acids, (ii) the occurrence of C_{15} and C_{17} aliphatic and branched-chain components and (iii) the distributions of the positional and geometric isomers of the monounsaturated C_{18} fatty acids. Fatty acids are by far the most abundant lipid components identified in solvent extracts from archaeological vessels and have been shown to comprise a wide range of different carbon number and various geometric and positional isomers which are present in fresh fats. Few attempts have been made previously to quantify the distributions of positional and geometric isomers in unsaturated fatty acids, but it has been established in this thesis that the distinctive distributions can be used to support other chemical criteria. Unfortunately fatty acid distributions in remnant fats usually appear to have suffered from decay and the original profile is often unrecognisable. The over-riding factor appears to be the relative susceptibility of different chemical components to decay. It is recommended that free fatty acid distributions are used only in combination with other characteristics in determining the origin of degraded fats on account of the following:

(i) The preferential decay of unsaturated components which are diagnostic in distinguishing between fresh dairy fats and raw and cooked meats (Matter *et al.*, 1989; Matter, 1992), often leads to the complete loss of polyunsaturated components and diminished abundances of the monounsaturated components due to their susceptibility to oxidative decay;

(ii) Shorter-chain fatty acids (< C_{12}) are lost preferentially due to their higher chemical reactivity and solubility (Balls, 1937); the decay of fresh dairy fats leads to the loss of the diagnostic short-chain components, resulting in a lipid profile which is indistinguishable from degraded adipose fat;

(iii) The $C_{14:0}$:$C_{17:0}$ ratio has been shown to be useful as a measure of decay, however, caution must be taken in using the proportion of $C_{14:0}$ in an extract as an indicator of origin, since it is more susceptible to loss by dissolution than the longer-chain $C_{16:0}$ and $C_{18:0}$ fatty acids;

(iv) The original $C_{16:0}$:$C_{18:0}$ fatty acid ratio is frequently altered due to the greater susceptibility of the lower carbon-number moiety to loss by dissolution, e.g. in groundwaters. This has been evidenced by the number of highly degraded archaeological extracts which comprise a significantly higher abundance of the free $C_{18:0}$ fatty acid component which does not correspond with the other chemical criteria studied. We have frequently observed remnant dairy fats with unexpectedly low abundances of $C_{14:0}$, which would indicate that the relative abundances of the $C_{16:0}$ and $C_{18:0}$ fatty acids does not reflect the original ratio. Thus the data indicate that extreme caution should be observed in using the ratios of free fatty acids. However, a reliable indicator of the original fatty acid ratio in remnant fats can be obtained where mid-chain ketones (C_{31}, C_{33} and C_{35}) are present in the residue;

(v) Although the overall abundance of the monounsaturated fatty acids is always depleted in the archaeological fats, in many cases there was sufficient $C_{18:1}$ remaining to enable the distributions of isomers to be determined. It is evident, however, that the *cis*-configured isomers have suffered from decay to a much greater extent than the *trans*-forms, with only the *cis*-Δ^9 remaining in low abundance in the archaeological extracts. For this reason, $C_{18:1}$ *cis/trans* ratios should not be considered a reliable criterion.

(vi) Remnant porcine fats have been found to contain the branched-chain and odd-carbon number fatty acids seen in ruminant fats which complicate their identification since their chemical composition resembles mixtures of ruminant and non-ruminant fats.

The appearance of these minor components is probably due to the contribution of exogenous fatty acids in their diet, e.g. waste meat or whey, from which fatty acids are transferred directly to the depot fats of the non-ruminant animals, and not the result of bacterial contamination post-burial.

vii) Variable preservation has been observed due to burial conditions, e.g. the fatty acid composition of extracts from Eton are less well preserved than in sherds from the same archaeological period excavated from Yarnton. Various factors such as soil type, climate, frequency of waterlogging, etc. may influence the preservation of organic residues. The loss of free fatty acids from ceramic sherds in alkaline and saline soil conditions can been attributed to the conversion of fatty acids to water soluble salts which would increase the chances of their being lost by leaching through groundwater inundation of the sherd during burial. Salts may also be formed by interactions between trace metals in the clay wall of the pot and absorbed lipid residues, as indicated in a test study whereby a coating of free fatty acids ($C_{18:1}$, $C_{16:0}$ and $C_{18:0}$) applied to copper was 50% converted to the copper salts in only 4 days; after 2 months conversion was complete (Schrenk, 1990).

The physico-chemical and biological processes leading to the loss of the major fatty acid components has been found to result in degraded animal fats from both ruminant and non-ruminant depot and ruminant dairy fats which are indistinguishable on the basis of fatty acid distributions alone. The data have indicated that great caution is required in interpretation of data from highly degraded fats due to the potential for alteration which provides misleading evidence. However, the distributions of geometric and positional isomers of the $C_{18:1}$ components have provided a reliable criterion for making distinctions between remnant ruminant and non-ruminant fats, even where decay has significantly altered the original lipid distributions. The incorporation of fatty acid contamination from bacteria or through the migration of soil lipids during decay in the burial environment is considered to be minor, as demonstrated through the laboratory decay of pure triacylglycerols absorbed in ceramic sherds.

8.3.2 The use of triacylglycerol distributions in determining species origin

Laboratory decay experiments carried out during the course of this study have indicated that triacylglycerols containing unsaturated fatty acyl moieties are more highly susceptible to decay than their saturated counterparts. In their intact form, ruminant dairy and adipose fats can be readily distinguished by a greater abundance of the lower carbon number triacylglycerols in dairy fats, however, the preferential loss of these shorter-chain moieties during decay has been shown to result in remnant fats which are very similar in composition. The susceptibility to decay of the diagnostic short-chain fatty acyl moieties compared to their long-chain counterparts is due to the fact that the short-chain fatty acid moieties are located primarily at the *sn*-3 position in triacylglycerols in natural fats (Brockerhoff *et al.*, 1966; Parodi, 1979, 1982) and will be more susceptible to release by enzymatic and chemical hydrolysis. These diagenetic alterations have been the main reason for our inability to identify dairy fat residues in pottery.

It is unclear whether our inability to detect bovine adipose fat residues amongst the archaeological assemblages is a true reflection of the fact that bovine fats were infrequently processed in pottery vessels. Since butchery marks provide strong evidence for the exploitation of bovines for their meat, it is possible that decay has altered the distributions of triacylglycerols in remnant bovine adipose fats so that they are indistinguishable from other ruminant fats. The main distinction between distributions in ovine and bovine adipose fats is the predominance of the C_{50} over the C_{52} component in bovine fats. However, preferential decay of lower carbon-number components may have reduced the relative abundance of the C_{52} component predominant in bovine fats, resulting in a lipid distribution which resembles an ovine fat. Despite this, one of the diagnostic characteristics of ovine fats is the high abundance of the C_{54} component, and this should still enable tentative distinctions to be made.

The susceptibility of triacylglycerols containing mono- and polyunsaturated moieties to oxidation is evident due to the absence of the majority of the unsaturated free fatty acids in the FAMEs prepared from the archaeological lipid extracts. The TLC separation of the saturated triacylglycerol fractions of the modern reference fats has therefore provided excellent reference material for comparison with the diagenetically altered fats. The

preferential loss of the unsaturated components is particularly significant in the more highly unsaturated fats. Indeed, the triacylglycerol distributions in degraded plant oils such as olive oil are completely transformed from their original distribution. However, this is not a significant problem as long as the pattern of decay is known and can be related to the original fat. The distinction between degraded olive oil and animal fats would in fact appear to be quite straightforward, based upon the comparison of the distributions of saturated triacylglycerol moieties. Consideration should perhaps be given to whether the higher saturation of dairy fats, e.g. butter, compared to either adipose or non-ruminant fats results in a bias in the types of residues we see in pottery vessels due to their different preservation potentials. The residues analysed for this study were selected partly on the basis that they contained intact triacylglycerols, however, it is often the case that residues are highly degraded and no longer contain intact acyl lipids. This is obviously a disadvantage for determining the origin of the remnant fat, since in combination with the isotopic analyses, the intact triacylglycerol distributions have proven an extremely diagnostic criterion.

8.3.3 The use of stable carbon isotope ratios in determining species origin

The $\delta^{13}C$ data have proven invaluable in distinguishing between fats of ruminant and non-ruminant origin and also between ruminant dairy and adipose fats. We have also been able to detect mixtures of ruminant and non-ruminant fats and provide semi-quantitative estimates of the different proportions of fats in mixtures based upon comparison with the data obtained from the theoretical mixing equation. However, despite the apparent reliability of $\delta^{13}C$ measurements based on the clustering of archaeological fats, it is essential to consider a range of chemical criteria in making assignments in order to enable accurate interpretations of the data.

During this work we have identified surprisingly few archaeological fats which do not clearly correspond to one or other of the fat types or to mixtures of fats when a range of chemical criteria are considered. The distortion of the original isotopic signal of the fat due to fatty acid contamination from the burial environment or microbial contamination and/or reworking of fatty acids is a consideration, however the $\delta^{13}C$ data from long-term laboratory decay of lamb fat and the minor contribution of bacterial fatty acids during the

decay of pure triacylglycerols have indicated that this should be minor. In addition, the fact that we observe a clear distinction between groups of archaeological fats from animals slaughtered ca. 5000 years ago, corresponding to the data for the reference fats, indicates the robust nature of the chemical signal and is extremely encouraging for the use of stable carbon isotope data in archaeological studies.

We have identified a shift in the ranges of $\delta^{13}C$ data for remnant fats from the same animal origins in the Stanwick and West Cotton assemblages. In fact, the mean of the data from the Late Saxon/early medieval pottery from West Cotton is ca. 1 to 2‰ more enriched in ^{13}C than the Iron Age/Romano-British data from Stanwick. Since this shift towards a higher proportion of ^{13}C is the opposite to that we would expect as a result of deforestation, it is hypothesised that the difference is due to fundamental changes in farming practice occurring between the Roman and Late Saxon periods in this region. The data suggest significant changes in the feeding regimes of the domesticated animal species which are borne out in the stable isotopic composition of their fats. The enrichment may have occurred due to the consumption of a higher proportion of cereals, increasing the fat and protein content in the diet, perhaps as a way of increasing milk output to cater for the expanding population at West Cotton. Indeed, the effect of feeding ruminant animals dietary supplements, has been seen in the analyses of the commercial animal fats which are relatively enriched in ^{13}C compared to the C_3 pasture-reared animals. In general, however, the data from the prehistoric sites appear to be quite comparable with the spread of the data obtained for the modern reference fats when the change of ca. 1.2‰ in atmospheric CO_2 which has occurred mainly since the onset of the Industrial Revolution has been taken into account. The data suggest there has been no significant change in the $\delta^{13}C$ value of atmospheric CO_2 prior to the Industrial Revolution, i.e. due to the effects of deforestation.

Distinctions between different fats at the same site are likely to be more reliable than comparisons between fats from different sites which should be treated with caution. This is particularly relevant when analysing remnant fats from different countries since large variations in $\delta^{13}C$ data in particular are likely, e.g. Woodbury *et al.* (1998) have seen differences of 0.5 to 1.0‰ for the $\delta^{13}C$ values of maize oils grown in Northern and Southern hemispheres, and it is likely that differences will exist between animal fats from

different geographical locations due to differences in native species of vegetation, environmental conditions and climate between continents which may result in differences in the $\delta^{13}C$ values of forage materials. Potential errors due to spatial isotopic changes are minimised by examining as many carbon sources as possible at a particular site and this is the basis of an ongoing project in our laboratory, to identify the isotopic compositions of different forage materials available in prehistory. It is recommended that animals and plants from the same locality as the archaeological fats should always be analysed as reference materials.

8.4 Future work

The identification of dairy fats as a dietary resource in prehistory

Significantly, by use of a combination of chemical criteria, the apparent enigma of the lack of significant numbers of milk fat residues in archaeological pottery has been resolved and a basis has been established for future studies of dairying as a agricultural practice amongst ancient peoples. Future work will focus on the use of this robust method of detecting dairying to obtain direct evidence for the earliest exploitation of ruminant animals for their milk in prehistory and to investigate the origins of the 'Secondary Product Revolution'. In the wider context, this technique can be applied to the study of dairying amongst ancient European and Near Eastern agriculturists, thereby allowing us to trace dairying to earlier dates and to study the exploitation of dairy products amongst populations believed to have been lactose-intolerant.

This work is currently the subject of a new research project being jointly funded by English Heritage and the Natural Environment Research Council. The project involves: (i) the analysis of at least 600 potsherds from large, well-chosen prehistoric assemblages, particularly aimed at the detection of the origins of dairying in the UK; (ii) the correlation of the occurrence of specific residues with the typological and stylistic attributes of the vessels from which they are derived, and (iii) the use of decay experiments (laboratory and field) involving milk and butter fats to investigate the robustness of the $\delta^{13}C$ values of the major fatty acids and the effect of different soil types on lipid decay. Carbohydrate and fatty acid components of diets will be analysed from key grasses, herbs and tree leaves

identified from the archaeobotanical site record (pollen and plant macrofossils) in order to establish the variation in isotopic composition of vegetation/forage available in prehistory.

Routing of dietary fat to non-ruminant body fats

Figure 8.21 compares the $\delta^{13}C$ values obtained for the reference fats and their diets. The $C_{16:0}$ and $C_{18:0}$ fatty acids in the adipose fats of non-ruminants are all significantly more enriched in ^{13}C relative to the fat components in their diet. The $\delta^{13}C$ values of the dietary fatty acid components of the chicken and goose are significantly different, however the tissue fatty acids are very similar. This suggests that the isotopic composition of the fat in the diets of these closely related species is not a significant factor in determining adipose fat composition. Further work involving the study of controlled-feeding experiments and isotopic analysis of the different biochemical fractions of the diet would enable a better understanding of the ways in which carbon from the diet is routed and/or scrambled during the formation of body fats. This work is currently being investigated as part of a case funded PhD in our laboratory, based upon the study of control-fed rats.

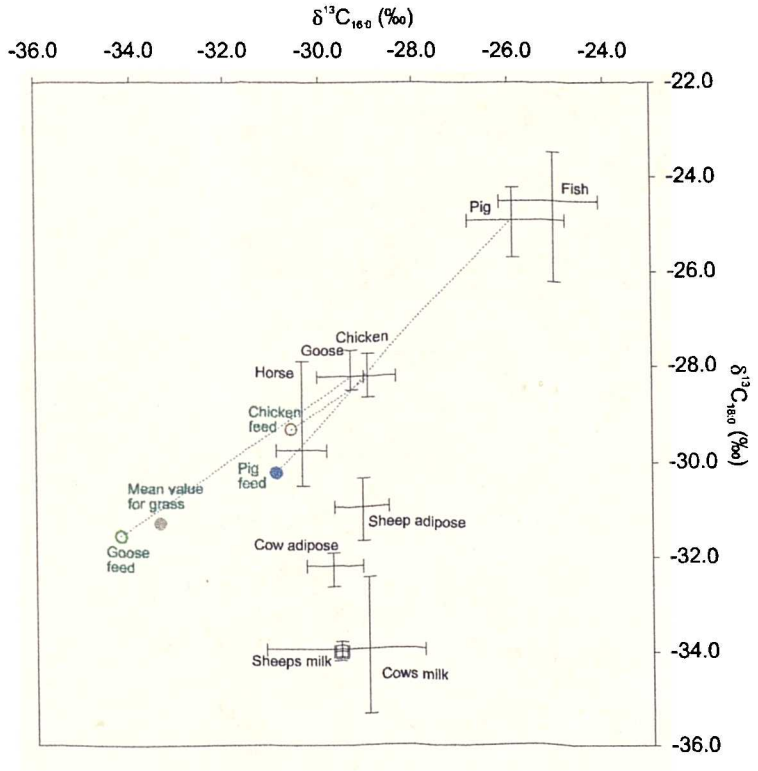


Figure 8.21 Ranges of $\delta^{13}C$ values for the $C_{16:0}$ and $C_{18:0}$ fatty acids in modern animal fats compared with the corresponding fatty acids in their diets. Error bars represent the range of $\delta^{13}C$ values obtained for the samples analysed, crossing at the arithmetic mean. The dotted lines indicate the relative ^{13}C enrichment of fatty acids in the non-ruminant body fats compared with fatty acids in their diet.

Stereospecific analysis of triacylglycerols

Promising results have been obtained from an initial study (Crossman, 1998) to investigate the use of APCI-MS to determine triacylglycerol positional isomers for use as a criteria in distinguishing between degraded fats. As a continuation of this work it would be interesting to determine the distributions of positional isomers in the saturated triacylglycerol fractions (separated by silver ion TLC) of modern fats for comparison with the archaeological data.

Characterisation of wax esters

Wax esters have been detected in a number of the residues analysed as part of this thesis, of which the origin is unknown, however, GC/MS analysis would enable the elucidation of their structures, facilitating the identification of the commodities mixed with the animal fats and enabling further insight into vessel function in antiquity.

Elucidation of components comprising the UCMs

Continuation of the work to identify fish residues in the archaeological record will include the analysis of laboratory-degraded fish oils. Decay experiments have already been initiated using cod liver oil absorbed in replica ceramic sherds. In order to identify the components present in the UCMs from the archaeological samples, total lipid extracts will be fractionated and analysed by GC/MS, and free fatty acids will be analysed by GC-C-IRMS in order to determine their origin.

The Grooved ware/pig fat association

The occurrence of remnant porcine adipose fats in Grooved ware vessels at both Yarnton and Upper Ninepence would not appear to be coincidence and may indicate a bias in the use of this vessel types or a preference for the exploitation of different species during different phases. The apparent increase in the occurrence of pigs in the Grooved ware phase at these sites is in keeping with Grooved Ware faunal assemblages elsewhere, for example, at Durrington Walls where the preponderance of young pig has been regarded as evidence for feasting (Wainwright and Longworth, 1971). Larger numbers of samples from Grooved ware assemblages need to be analysed in order to confirm the association between Grooved ware and the exploitation of pigs for their meat and fat.

CHAPTER 9
Analytical Protocol and Instrumental Conditions

9.1 Experimental

Figure 9.1 illustrates the range of analytical techniques employed in this study. The methodologies are described in more detail below.

9.1.1 Solvent extraction of lipid residues

Lipid analyses of potsherds were performed using our established protocol, whereby approximately 2 g samples were taken and their surfaces cleaned using a modelling drill to remove any exogenous lipids (e.g. soil or finger lipids due to handling). The samples were then ground to a fine powder, accurately weighed and a known amount (20 μg) of internal standard (*n*-tetratriacontane) added. The lipids were extracted with a mixture of chloroform and methanol (2:1 v/v). Following separation from the ground potsherd the solvent was evaporated under a gentle stream of nitrogen to obtain the TLE.

Carbonised surface residues were scraped from the surfaces using a scalpel and powdered using a pestle and mortar. The extractions proceeded as for the powdered sherds.

9.1.2 Solvent extraction of modern reference fats

Adipose tissue (*ca* 2 g) was placed in a conical flask with a mixture of chloroform and methanol (2:1 v/v; 20 ml) and sonicated (2 x 15 mins). The extract was filtered through sodium sulphate to remove residual water, and the bulk of the solvent removed using a rotary evaporator. The remaining solvent was removed under a gentle stream of nitrogen and the TLE stored at -20 °C until required for analysis.

9.1.3 Preparation of trimethylsilyl derivatives

Portions of the total lipid extracts were derivatised using *N,O*-bis(trimethylsilyl) trifluoroacetamide (20 μl; 70°C; 45 mins; T-6381; Sigma-Aldrich Company Ltd., Gillingham, UK) and analysed by GC and GC/MS.

9.1.4 Acid/neutral separations

The free fatty acids were separated from the neutral fraction using a 500 mg/2.8 ml aminopropyl Bond Elute solid phase column (AHO-1210-2041; Phenomenex, Macclesfield, Cheshire, UK) conditioned with hexane (2 ml). A portion of the total lipid

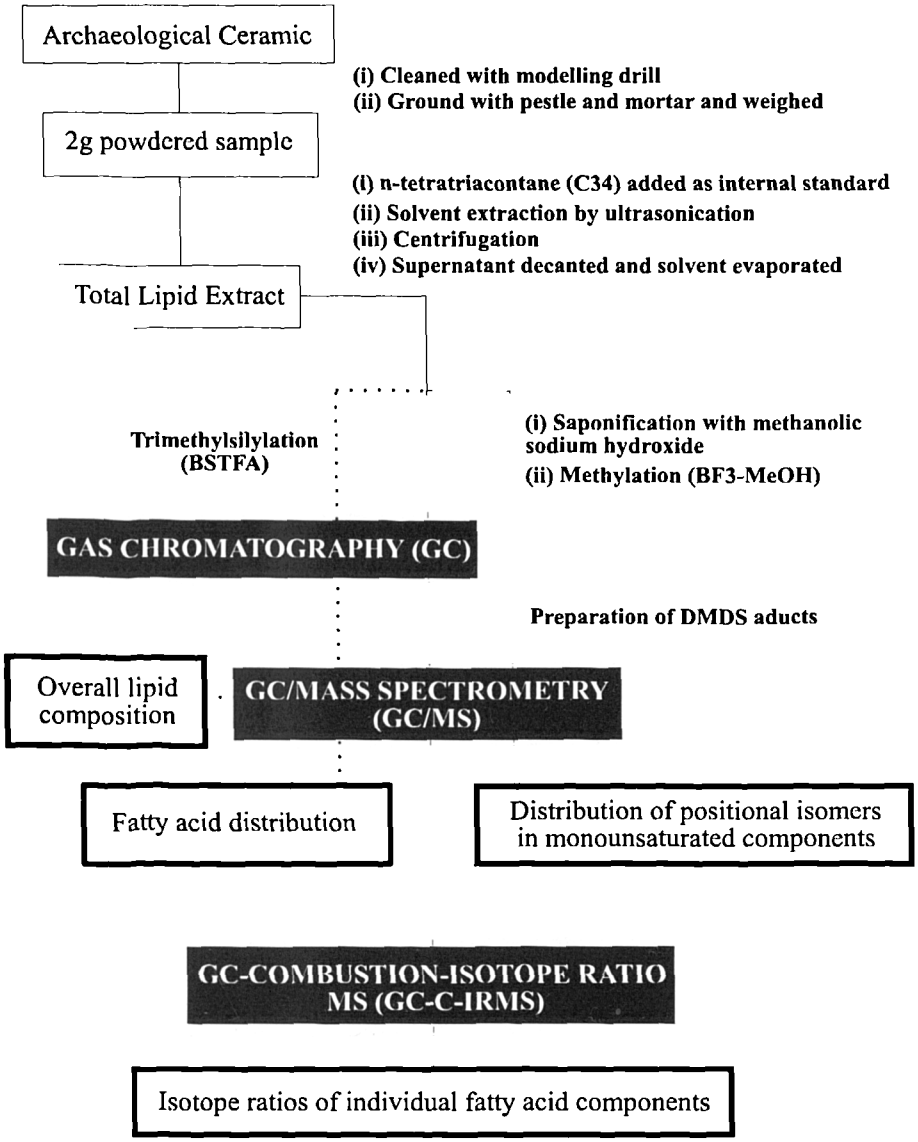


Figure 9.1 Flow diagram illustrating analytical methods followed. Procedures are detailed in the text.

extract in dichloromethane (DCM)/isopropanol (2:1 v/v) was applied to the column and the neutral fraction eluted with further application of DCM/isopropanol (5 ml). The acid fraction was eluted by the addition of formic acid in diethyl ether (2%; 5ml). The two fractions were collected in separate vials and the solvents removed under N_2.

9.1.5 Saponification of total lipid extracts

Methanolic sodium hydroxide (5% v/v) was added to the TLE and heated at 70°C for 1 hour. Following neutralisation, lipids were extracted into hexane and the solvent reduced by rotary evaporation.

9.1.6 Preparation of fatty acid methyl ester derivatives

FAMEs were prepared by reaction with BF_3-methanol (14% w/v; 2 ml; B-1252; Sigma-Aldrich, Gillingham, UK) at 70°C for 1 hour. The methyl ester derivatives were extracted with diethyl ether and the solvent removed under nitrogen. No kinetic isotope effect is associated with this derivatisation because the reaction is rapid and quantitative with regard to the carbonyl (Rieley, 1994). FAMEs were re-dissolved into hexane for analysis by GC and GC-C-IRMS.

9.1.7 Preparation of dimethyl disulphide derivatives

A portion of FAMEs in hexane (100 ul) was transferred to a small screw-capped vial to which dimethyl disulphide (100 ul; 1655962500; Acros Organics, NJ, USA) was added with iodine in diethyl ether (6% w/v; 2 drops). The mixture was covered and left over night at 45 °C. Aqueous sodium thiosulphate (5%; 500 ul) was added and the solution shaken until clear in colour and the DMDS derivatives extracted with hexane (3 x 2 ml). The extracts were combined and the hexane removed under N_2 prior to analysis by GC/MS.

9.1.8 Silver ion adsorption thin-layer chromatography

The neutral lipids were separated using an NH_2 animo propyl Bond Elute column, as described in Section 9.1.4, and fractionated into triacylglycerol classes on glass TLC plates (20 x 20 cm) coated to a thickness of 0.3 mm with silica gel impregnated with 20% silver nitrate (2801120; Alltech Associates Inc., Deerfield, IL, USA) and activated at 120°C for 1 hr. Triacylglycerol mixtures were applied in hexane and developed using the solvent

system chloroform:toluene:ethanol (70:30:0.25). The plates were sprayed with 0.2% ethanolic dichlorofluoroscein and visualised under ultraviolet light. Bands were scraped into 2 ml vials prior to filtration through 4 cm silica gel columns using diethyl ether: methanol (90:10) as the elution solvent. Tricaprin (20 μg) was added to each fraction to enable relative abundances of each triacylglycerol class to be determined. Fractions were analysed by HTGC and GC/MS.

9.2 Instrumentation

9.2.1 Gas chromatography

GC analyses were performed on a Hewlett Packard 5890 gas chromatograph (Hoofddorp, The Netherlands), coupled to an Opus V PC using HP Chemstation software, which provided instrument control, data acquisition and post-run data processing facilities. Total lipid extracts were introduced by on-column injection into a 15 m x 0.32 mm i.d. fused silica capillary, coated with DB1 stationary phase (immobilised dimethyl polysiloxane, 0.1 μm film thickness; 123-1011; J & W Scientific, Folsom, CA, USA). The temperature programme consisted of a 2 minute isothermal hold at 50°C followed by a ramp from 50 to 350°C at 10°C min^{-1}. The temperature was then held at 350°C for 10 min. FAMEs were analysed using a 50 m x 0.32 mm i.d. WCOT fused silica capillary, coated with CP-Wax-52 CB stationary phase (polyethylene glycol, 0.2 μm film thickness). The temperature programme consisted of three ramps from 40 to 150°C at 15°C min^{-1}, from 150 to 220°C at 4°C min^{-1} and from 220 to 240°C at 15°C min^{-1} remaining at 240°C for 15 min. In both cases, hydrogen was used as carrier gas (head pressure 10 psi) and flame ionisation detection was used to monitor the column effluent.

9.2.2 Gas chromatography/mass spectrometry

GC/MS analyses were performed using a Finnigan 4500 quadrupole mass spectrometer (Finnigan MAT GmbH, Bremen, Germany) directly coupled to a Carlo Erba 5160 Mega series GC with on-column injection. Operating conditions were as follows: ion source, 170°C; emission current, 400 μA and electron energy, 70 eV. The GC-MS interface was maintained at a temperature of 350°C. Spectra were recorded over the range *m/z* 50-850 every 1.5 s. Data were acquired and processed using an INCOS data system. GC operating

conditions were the same as those described in Section 9.2.1, except for the DMDS derivatives which were analysed on the 50m fused silica capillary CP-Wax-52 CB column using a temperature programme which consisted of two ramps from 50 to 150°C at 15°C min^{-1}, from 150 to 250°C at 4°C min^{-1} remaining at 250°C for 20 min. In all cases, helium was used as carrier gas.

9.2.3 Gas chromatography-combustion-isotope ratio mass spectrometry

Analyses were carried out using a Varian 3400 gas chromatograph (Varian Associates Inc., Walnut Creek, CA) attached to a Finnigan MAT Delta-S isotope ratio mass spectrometer (Finnigan MAT GmbH, Bremen, Germany) *via* a modified Finnigan MAT (Pt/CuO) combustion interface. Removal of water after combustion was facilitated by Nafion tubing (Perma Pure Products Inc., Toms River, NJ) and standardisation of runs was achieved with six portions of CO_2 gas of known $\delta^{13}C$ value ($\delta^{13}C_{(CO_2)}$ = -31.80 ‰) injected directly into the ion source of the mass spectrometer. The GC column used was a 50 m x 0.32 mm i.d. WCOT fused silica capillary, coated with CP-Wax-52 CB stationary phase (polyethylene glycol, 0.2 μm film thickness). The temperature programme consisted of three ramps from 40 to 150°C at 15°C min^{-1}, from 150 to 220°C at 4°C min^{-1} and from 220 to 240°C at 15°C min^{-1} remaining at 240°C for 15 min and helium was used as carrier gas. The Cu/Ni/Pt reactor was maintained at a temperature of 860°C. The mass spectrometer source pressure was $6x10^{-6}$ mbar. Samples were injected *via* a septum equipped temperature programmable injector (SPI). Carbon isotope ratios were expressed relative to VPDB.

To determine $\delta^{13}C$ values of the fatty acids from their corresponding FAME, a correction was made for the carbon atoms incorporated on derivatisation. This was achieved by using the mass balance equation of Jones *et al.* (1991) and assuming there is no isotope fractionation associated with the derivatisation procedure. Hence,

$$\text{for } C_{16:0} \text{ fatty acid } 16.D_{FFA} = 17.D_{FAME} - X$$
$$\text{for } C_{18:0} \text{ fatty acid } 18.D_{FFA} = 19.D_{FAME} - X$$

where D_{FFA} = $\delta^{13}C$ value of the free fatty acid, D_{FAME} = $\delta^{13}C$ value of the FAME and X = $\delta^{13}C$ value of the derivatising carbon (-31.4‰).

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APPENDIX 1

APPENDIX 1. RESULTS OF SCREENING ARCHAEOLOGICAL AND ETHNOGRAPHIC VESSELS

Table 1. Description of samples, lipid content and description of lipid components in sherds from West Cotton, Northamptonshire (Late Saxon/early medieval). Assignments of animal fat origins given in *Commodities Processed* have been made mainly previously on the basis of free fatty acid distributions by Charters (1996).

Sherd no. and ware type	Vessel form	Date	Visible evidence	Lipid content ($\mu g\ g^{-1}$)	Lipid components present	Commodities processed
RP2 BODY*	Shelly ware jar	1150-1225	Smoke-blackened lower body	202	DAF; C_{26}, C_{27}, C_{28} OH; C_{44}-C_{52} WE; C_{29} K	Beef fat (trace leaf waxes)
RP4 RIM*	Shelly ware jar		Outer body surface blackened, more concentrated towards the base, base scorched reddish orange	1008	DAF; C_{27}, C_{29} AL; C_{26},C_{27},C_{28} OH; C_{29},C_{31},C_{33} K	Pork fat (trace leaf waxes)
RP6 RIM*	Dish			402	DAF, K (C_{31},C_{33},C_{35})	Animal products (trace leaf waxes)
RP7 BODY*	St. Neots ware jar	1100-1150	Lower body and exterior base smoke-blackened; inner base-pad burnt	57	DAF; C_{29} AL; C_{26}, C_{28}, C_{29} OH; C_{29} K	Animal products and cabbage (*Brassica oleracea*)
RP10 BODY*	Shelly ware bowl			28	DAF	Animal products

Table 1. Continued.

RP13 BODY*	Lyveden A ware jar	1250-1300	Heavily blackened base-pad	122	DAF; C_{29},C_{31},C_{33},C_{35} AL; C_{26},C_{28},C_{30},C_{32} AL; C_{42}-C_{54} WE, C_{29},C_{31} K	Animal products, cabbage and leek (*Allium porrum*)
RP16 BODY*	Lyveden A ware jar	1225-1250	Body (shoulder to base-pad) extensively smoke blackened	3474	DAF (trace WE, AL, OH components)	Lamb fat
RP22 BASE*	Lyveden A ware jar	1250-1300	Lower body and base evenly blackened	26	DAF; C_{29},C_{31} AL; C_{26},C_{28} OH;	Beef fat and leaf wax
RP28 BASE/ BODY*	Shelly ware 'top hat' vessel	1100-1150	Exterior patchily burnt and sooted	269	DAF; C_{29},C_{31} AL; C_{24},C_{26},C_{28},C_{29},C30 AL; C_{44}-C_{48} WE; C_{29} K	Lamb fat and leaf wax
RP30 BASE/ BODY*	Oolitic courseware jar	1150-1225	Progressively more blackened towards base	1840	DAF, C_{29}, C_{31} AL; C_{26}, C_{28}, C_{29} OH; C_{29} K	Animal products (trace leaf waxes)
RP50 BODY*	S/J (probably Thetford ware)			4163	DAF	Animal product
RP53 U. BODY*	Shelly ware 'top hat' vessel	1100-1150	Most outer body blackened; patches of sooting; base-pad scorched orange	351	DAF	Animal product
RP60 BASE*	Shelly ware 'top hat' vessel	1150-1225	Patches of blackening on outer surfaces; inclusions leached out on lower half of inner body and base-pad	1194	DAF	Lamb fat

Table 1. Continued.

RP61 BODY*	Shelly ware jar	Late Saxon	Blackened exterior base-pad; inner surface inclusions leached out of base-pad and lower 5 cm of inner body surface	22	DAF (trace WE, AL, OH components)	Beef fat (trace leaf waxes)
RP71 BASE*	Shelly ware 'top hat' vessel	1100-1150	Outer lower body blackened and smoked, darker towards base; outside of rim heavily blackened and sooted	1076	DAF	Lamb fat
RP72 RIM*	St. Neots ware bowl	1100-1150	Outer body blackened except top of rim and upper half of spout; some outer surface blackening	3868	DAF (trace WE, AL, OH components)	Animal product (trace leaf waxes)
RP73 RIM*	St. Neots ware bowl	1150-1225	Totally blackened outer surface	2731	DAF; C_{27},C_{29} AL; C_{26},C_{28},C_{30},C_{32} OH; C_{46},C_{48} WE	Mainly animal product (Note: Some beeswax in base sherd)
RP78 RIM*	Shelly ware jar		Outer surface blackened; scorched red outer base-pad	4840	DAF; C_{25}-C_{31} AL; $C_{31:1}$, $C_{33:1}$ AE; C_{24},C_{26},C_{28},C_{30} OH; C_{42}-C_{48} WE	Mainly animal product (Note: Some beeswax in base and body sherds)
RP81 BODY*	St Neots ware jar	Late Saxon		2033	DAF (trace WE, AL, OH components)	Animal product (trace leaf waxes)

Table 1. Continued.

RP82 RIM*	Furnells 'Top Hat' pot			1793	DAF (trace WE, AL, OH components)	Animal product (trace leaf waxes)
RP83 RIM*	Lyveden B fish dish Internally glazed			1263	DAF	Animal product
RP85 RIM*	Furnells Lyveden A jar		Located in cess pit	1101	DAF	Animal product
RP86 BODY*	Furnells 'Top Hat' pot			428	DAF	Animal product
RP87 RIM*	Furnells Lyveden A jar, very large vessel		Located in cess pit	315	DAF	Animal product
RP88 BODY*	Furnells Lyveden A jar			28	DAF (trace WE, AL, OH components)	Animal product (trace leaf waxes)
RP89 L. BODY*	Furnells Manor 'cooking pot'	Early Saxon		1918	DAF,K (C_{29},C_{30},C_{31}, C_{32},C_{33},C_{34},C_{35})	Animal product
RP91 RIM*	Furnells site-reused as griddle?			1169	DAF	Animal product
RP93 SPOUT*	St Neots ware 'spouted bowl'			3326	DAF	Animal product
RP94 RIM/BODY*	St Neots ware 'spouted bowl'	Late Saxon		2686	DAF	Animal product
WC30*	'Top Hat' pot			1079	DAF	Animal product

Table 1. Continued.

* Extracts analysed by GC-C-IRMS

Key

C_n	No. of carbon atoms	WE	Wax ester
AL	Alkane	AE	Alkene
K	Mid-chain ketone	OH	Alcohol
DAF	Degraded animal fat (includes mono-, di- and triacylglycerols)	*	Extracts analysed by GC-C-IRMS

Footnote: Extractions and analyses have been performed as described in Chapter 8 and lipid content is measured in micrograms per gram dry weight of sherd ($\mu g\ g^{-1}$). Description of the lipid components and commodities present in each extract are given. Components which could not be identified on the basis of GC retention time alone were elucidated by GC/MS analysis.

Table 2. Description of samples, lipid content and description of lipid components present in sherds from Stanwick, Northamptonshire (Iron Age/Romano-British).

Sherd no. and ware type	Vessel form	Date	Context	Other finds	Lipid content ($\mu g\ g^{-1}$)	Lipid components present
Romano-British						
Grogged ware						
ST 190 Body*	Channel-rim jar	C2	Fill of pit	AB	952	DAF, K (C_{29}), CH
ST 190 Rim*	Channel-rim jar	C2	Fill of pit	AB	3990	DAF, K (C_{29}), CH
ST 193 Base*	Bowl	C1-3+/LC1-2	Lower plough soil/fill of ditch	M,Sa,AB,S,St,Ir	130	DAF, OH, WE, K (C_{29}), Sit
ST 194*	Wide bowl	C1-4	Lower plough soil	G,M,AB,TB,St,Sl	87	DAF,OH,WE
ST 197*	Deep jar	C1	Fill of pit	AB,Ir	615	DAF, CH
Shelly ware						
ST 206 Body*	Channel-rim jar	C1-2/LC1-2	Fill of ditch-sand, gravel, grey clay	AB,Fl,FC	130	DAF
ST 206 Rim*	Channel-rim jar	C1-2/LC1-2	Fill of ditch-sand, gravel, grey clay	AB,Fl,FC	907	DAF, K (C_{31},C_{33},C_{35})
ST 208*	Dish	C4	Occupation layer-burnt ash, charcoal	Sa,S	1419	DAF, CH
ST 210 Rim-body*	Jar		Occupation layer-burnt ash, charcoal	Sa,S	165	DAF, CH
ST 211*	Jar		Courtyard layer-burnt ash, charcoal	AB,M,G,Sa,TB,Sl, Ir	1398	DAF
ST 212*	Flanged bowl		Courtyard layer-burnt ash, charcoal	AB,M,G,Sa,TB,Sl, Ir	627	DAF
ST 215*	Channel-rim jar	MC1-LC1/C4	Lower plough soil	AB,S,Sa,Ir	423	AF, OH, CH

Table 2. Continued.

Grey ware						
ST 226 Base*	Dish	LC1-2	Fill of ditch	AB,Sl,S	425	DAF, OH, K (C_{31},C_{33},C_{35})
Mortaria						
ST 262 Rim*	Mortaria	240-400 AD		POT,B	22	DAF
Iron Age		*ca.* 500-1000BC				
ST 152*			Fill of ditch	POT,AB,Sl,S,St	58	DAF
ST 153 Rim*	Jar		Fill of ditch	POT,AB,Sl,S,St	611	DAF (well preserved)
ST 156*	Large jar		Layer	POT,AB	490	DAF (well preserved)
ST 160*	Small jar		Fill of ditch	POT,AB,Sl	720	DAF, K (C_{31},C_{33},C_{35})
ST 161 Body*	Small jar		Fill of ditch	POT,AB	378	DAF

Key:

Other finds:

POT	Potsherds	S	Shell
AB	Animal bone	M	Mortaria
Sl	Slate	G	Glass
St	Stone	TB	Tile and brick
Sa	Samian ware	Ir	Iron
		Fl	Flint
		FC	Fired clay

Lipid components:

DAF	Degraded animal fat	AE	Alkenes
OH	Alcohols	CH	Cholesterol
K	Mid-chain ketones		
WE	Wax esters		
Sit	Sitosterol		
AL	Alkanes		

* Extracts analysed by GC-C-IRMS

Table 3. Description of samples, lipid content and description of lipid components present in sherds from Fuller's Hill, Great Yarmouth.

Sherd no.	**Lipid content ($\mu g\ g^{-1}$)**	**Lipid components present**
GY 197	Unquantified	Unresolved complex mixture (UCM)
GY 217	Unquantified	UCM
GY 304	62	Free fatty acids(including C_{17} branched- and straight-chain components), di- and minor triacylglycerols, possibly some wax esters present
GY306	Unquantified	UCM
GY 349	112	Free fatty acids(including C_{17} branched- and straight-chain components), di- and minor triacylglycerols
GY 424A	Unquantified	UCM
GY 457A	Unquantified	UCM, trace free fatty acids and intact triacylglycerols

Table 4. Description of samples, lipid content and description of lipid components present in sherds from Wickham Bonhunt, Essex (Middle Saxon and Romano-British).

Sherd no.	Date	Ware type and information	Lipid content ($\mu g\ g^{-1}$)	Lipid components present
Box no. 6 (HH/30/A/6) 1986.62.1.6				
1*	650-899 AD Middle Saxon	Ipswich ware cooking pot (rim)	51	DAF
2*	650-899 AD Middle Saxon	Ipswich ware cooking pot (rim)	51	DAF
6	650-899 AD Middle Saxon	Ipswich ware cooking pot (rim)	88	DAF
7*	650-899 AD Middle Saxon	Ipswich ware cooking pot (rim)	93	DAF
Box no.5 (HH/30/A/5) 1986.61.42				
3*	43-1149 AD Romano-British	Handmade earthenware, grass tempered	130	DAF
5	43-1149 AD Romano-British	Handmade earthenware, grass tempered	0	
10	43-1149 AD Romano-British	Cooking pot charred on outside, St Neots Ware	45	DAF
11	43-1149 AD Romano-British	Handmade earthenware, grass tempered	0	
13*	43-1149 AD Romano-British	Handmade, shell-tempered	676	DAF
15*	43-1149 AD Romano-British	Handmade, grass tempered	1427	DAF; C_{31},C_{33},C_{35} K
Box no. 8 (HH/30/A/8) 1986.62.1.2				
4	650-899 AD Middle Saxon	Earthenware and Ipswich ware 'cooking pots' with sagging bases	0	
12*	650-899 AD Middle Saxon	Earthenware and Ipswich ware 'cooking pots' with sagging bases	155	DAF; C_{31},C_{33},C_{35} K
14*	650-899 AD Middle Saxon	Earthenware and Ipswich ware 'cooking pots' with sagging bases	27	DAF
17	650-899 AD Middle Saxon	Earthenware and Ipswich ware 'cooking pots' with sagging bases	Trace	

Table 4. Continued.

19*	650-899 AD Middle Saxon	Earthenware and Ipswich ware 'cooking pots' with sagging bases	52	DAF; C_{31},C_{33},C_{35} K
20	650-899 AD Middle Saxon	Earthenware and Ipswich ware 'cooking pots' with sagging bases	0	
Box no. 13 (HH/30/B/13) 1986.62.1.1				
8*	650-899 AD Middle Saxon	Handmade earthen and vegetable tempered wares	1134	DAF
16*	650-899 AD Middle Saxon	Handmade earthen and vegetable tempered wares	331	DAF
18	650-899 AD Middle Saxon	Handmade earthen and vegetable tempered wares	1417	DAF

* Extracts analysed by GC-C-IRMS

Table 5. Description of samples, lipid content and description of lipid components present in sherds from Botai, Kazakhstan.

Sherd no.	Sample type	Lipid content ($\mu g\ g^{-1}$)	Lipid components present
PACKON I (No. 2)*	Pot	513	Free fatty acids, including $C_{14:0}$, $C_{15:0}$, $C_{16:0}$, $C_{17:0}$. $C_{18:0}$ and $C_{18:1}$; $C_{16:0}$ and $C_{18:0}$ monoacylgylcerols in trace amounts
PACKON I (No. 2)	Carbonised Residue	0	
PACKON II (No. 3)	Pot	0	
PACKON II (No. 3)*	Carbonised Residue	2955	Free fatty acids, including $C_{14:0}$, $C_{15:0}$, $C_{16:0}$, $C_{17:0}$. $C_{18:0}$ and $C_{18:1}$
PACKON III (No. 4)	Pot	0	
PACKON III (No. 4)*	Carbonised Residue	835	Free fatty acids, including $C_{14:0}$, $C_{15:0}$, $C_{16:0}$, $C_{17:0}$. $C_{18:0}$ and $C_{18:1}$; mono-, di- and triacylgylcerols in trace amounts
PACKON VII/ N21 (No. 6)*	Pot	349	Free fatty acids, including $C_{14:0}$, $C_{15:0}$, $C_{16:0}$, $C_{17:0}$. $C_{18:0}$ and $C_{18:1}$; mid-chain ketones
PACKON XVIII (No. 1)*	Pot	99	Free fatty acids, including $C_{14:0}$, $C_{15:0}$, $C_{16:0}$, $C_{17:0}$. $C_{18:0}$ and $C_{18:1}$; mono-, di- and triacylgylcerols (C_{44}-C_{54})
N26 (No. 7)*	Pot	351	Free fatty acids, including $C_{14:0}$, $C_{15:0}$, $C_{16:0}$, $C_{17:0}$. $C_{18:0}$ and $C_{18:1}$; mono-, di- and triacylgylcerols (C_{44}-C_{54}); mid-chain ketones

* Extracts analysed by GC-C-IRMS

Table 6. Description of samples, lipid content and description of lipid components present in sherds from Yarnton Cresswell Field, Oxfordshire (Early-mid Iron Age).

Sherd no.	Fabric type	Description	Lipid content ($\mu g\ g^{-1}$)	Lipid components present
101*	SG4	Lower body wall	288	FFA, C_{31},C_{33},C_{35} K
102	SA4	Body sherd	trace	
103	SA4	Lower body wall	trace	
104	SA3/4	Rim-type, slack shouldered jar?	13	DAF
105*	SN5	Rim-type, slack shouldered jar?	50	FFA, C_{31},C_{33},C_{35} K
106	SP3	Rim-type, tripartite jar	0	
107	SP4	Body sherd	trace	
108	LS4	Body sherd-limescale	21	FFA
109	SA4	Body sherd-limescale	0	
110	AS3	Body/base	trace	
111	AS4	Body	8	DAF, WE, OH
112*	GS4	Body	130	DAF
113*	SC5	Rim, barrel-shaped jar	85	DAF
114*	AG3	Body	11	DAF
115	SG5	Body	0	
116	SP4	Body/base	0	
117	SG4	Rim, slack shouldered jar	6	FFA
118*	SA5	Body	105	DAF, C_{31},C_{33},C_{35} K
119*	SL4	Body	15	DAF
120	SG4	Body/base	trace	
121*	AN3	Body	41	DAF
122	AI3	Body	trace	
123	AP3	Rim, barrel-shaped jar	0	
124	AN3	Body/base	trace	
125	SA4	Handle/shoulder	trace	
126*	GSA4	Body, bowl	161	DAF
127*	GSA4	Base, slack shouldered jar	44	DAF
128*	GSA4	Body, slack shouldered jar	15	DAF
129*	GSA4	Rim, slack shouldered jar	142	DAF
130*	SG4	Base	61	DAF
131	PAS3	Body	0	
132	CS3	Rim, jar	trace	
133	CQ3	Rim, jar	0	
134	SG4	Body	0	
135	SA4	Body FT DEC	6	Degraded beeswax?-poorly preserved
136	SP4	Body	8	DAF

Table 6. Continued.

137*	SG3	Rim	411	DAF
138	SA5	Rim, slack shouldered jar	5	FFA
139	LS4	Body	trace	
140	CS3	Body	trace	
141*	AN3	Body	75	DAF
142	SN4	Rim, barrel-shaped jar	41	Degraded beeswax, DAF, C_{31},C_{33},C_{35} K
143	SA4	Shoulder, slack shouldered jar	trace	
144*	SP4	Shoulder	28	DAF
145	SG3	Body	trace	
146*	SG3	Rim, tripartite jar	211	DAF
147	AN2	Body	5	FFA
148*	AN2	Body	27	DAF
149*	AN2	Body	114	DAF, C_{31},C_{33},C_{35} K

* Extracts analysed by GC-C-IRMS

Note: *fabric* is defined in terms of the first and second most important inclusion types (indicated by letters) followed by a numeric indicator of fineness, on a simple scale from 1 (very fine) to 5 (very coarse). The alpha codes are as follows:

A quartz sand
B 'black'sand (glauconite)
C calcaereous sand/grit
F flint
G grog
I oxide minerals
L limestone
M mica
N none visible
P clay pellets
Q large angular quartz(ite)
R rock-various
S shell
T fossil shell
U ironstone ooliths
V vegetable/organic
W uncertain white inclusions
Z indeterminate voids

Table 7. Description of samples, lipid content and description of lipid components present in sherds from Yarnton Floodplain, Oxfordshire (Neolithic and Bronze Age).

	Date/description	**Lipid content ($\mu g\ g^{-1}$)**	**Description**
YFPB 96			
1*	Mid-late Neolithic Pet ware	99	DAF (high MAGs)
2	Mid-late Neolithic Pet ware	5	-
3	Mid-late Neolithic Pet ware	0	-
4*	Mid-late Neolithic Pet ware	58	DAF (high MAGs)
5*	Mid-late Neolithic Fengate ware	163	DAF, C_{31},C_{33},C_{35} K (high MAGs)
10	Late Neo Grooved ware	Trace	-
11	Grooved ware	9	-
12	Grooved ware	Trace	-
13	Late Neo Grooved ware Durrington Walls	Trace	-
14	Grooved ware	6	-
15	Grooved ware	14	C_{16} MAG, DAG high
16	Grooved ware	8	-
17	Biconical urn, Early Bronze Age (EBA)	Trace	-
18	Biconical urn, EBA	Trace	-
19	Biconical urn, EBA	0	-
20	Biconical urn, EBA	0	-
21	Biconical urn, EBA	28	DAF, C_{31},C_{33},C_{35} K
22	Biconical urn, EBA	Trace	-
23*	Biconical urn, EBA	47	DAF
24	Grooved ware Durrington Walls late Neolithic, some with burnt residues	10	DAF, C_{31},C_{33},C_{35} K
25	Grooved ware Durrington Walls and Clacton	0	-
26	Early/middle Bronze Age	0	-
27	Middle Bronze Age Deverel-Rimbury	0	-
28	Mid-late Neolithic Pet ware	0	-
30*	Mid-late Neolithic Pet ware	26	DAF
31*	Mid-late Neo Mortlake ware	358	DAF
YWRF			
32	Mid-late Neo Mortlake ware	21	FFA
YCE 93			-

Table 7. Continued.

33	Beaker	104	FFA
34	Beaker	11	FFA
35	Fengate Ware	trace	-
36	Late Bronze Age	0	-
YFPWB95			
37	Beaker domestic ware	0	-
YFP92			
38*	Late Neolithic Grooved ware	35	DAF
YFPB95			
39	Late Bronze Age	trace	-
40	Mid-late Neolithic Fengate Ware	46	DAF, C_{31},C_{33},C_{35} K
YCF95			
41*	Early-mid Bronze Age	267	DAF
42	Early-mid Bronze Age	0	-
43*	Domestic beaker	9	DAF
YFP92			
44	Biconical urn miniature vessel.	47	DAF
46a	Biconical urn	trace	-
46b	Biconical urn	169	FFA
47	Biconical urn	trace	-
48	Beaker	15	-
49*	Beaker	150	DAF
50*	Beaker	247	DAF

* Extracts analysed by GC-C-IRMS

Table 8. Description of samples, lipid content and description of lipid components present in sherds from Eton Lake End Road, Oxfordshire (Late Neolithic-Early Bronze Age).

Sherd no.	Context no.	Small find no.	Description		Lipid components present
NRA1*	959	2054	Mortlake ware, rim/shoulder fragment	218	DAF, C_{31},C_{33},C_{35} K
NRA2*	959	2041-8	Complete profile	52	DAF
NRA2*	959	2041-11		72	DAF
NRA2*	959	2041-12		55	DAF
NRA2*	959	2041-rim		55	DAF
NRA3	1224	2272	Mortlake, rim/shoulder	trace	DAF
NRA4*	1224	2271	Mortlake, rim/shoulder	345	DAF
NRA5	1224	2208	Mortlake, rim/shoulder	trace	DAF
NRA6	1223	2386	Mortlake, rim/shoulder	0	
NRA7	1433	2397	Mortlake, rim/shoulder	0	
NRA8	606	2119	Multiple samples	0	
NRA8	606	2149		0	
NRA8*	606	2163		242	FFA
NRA8*	606	2164		170	DAF
NRA8	606	2165		0	
NRA8*	606	2166		89	DAF, C_{31},C_{33},C_{35} K
NRA9	606	1135	Rim/shoulder (washed)	58	DAF
NRA10*	529	865	Rim/shoulder (washed)	1178	DAF, C_{31},C_{33},C_{35} K
NRA11	685	571	Multiple samples	8	DAF
NRA11	685	950		trace	
NRA11	685	600		trace	
NRA11*	685	601		35	DAF
NRA11	685	1011		0	
NRA12	1066	2017	Rim/shoulder (washed)	trace	
NRA13*	685	772	Base sherd (limescale)	53	FFA, C_{31},C_{33},C_{35} K

* Extracts analysed by GC-C-IRMS

Table 9. Description of samples, lipid content and description of lipid components present in sherds from Eton Rowing Lake, Oxfordshire (Early Neolithic).

Sherd no.	Area	Vessel type	Sherd type	Lipid content ($\mu g\ g^{-1}$)	Lipid components present
DBC 1*	A6	Plain bowl	Rim	131	DAF
DBC 2	A6	Carinated bowl	Rim	4	DAF
DBC 3*	A6	Carinated bowl	Rim	36	DAF
DBC 4	A6	Plain bowl	Rim	0	
DBC 5	A10	Carinated bowl	Rim	trace	
DBC 6	A10	Carinated bowl	Shoulder	0	
DBC 7*	A10	Carinated bowl	Shoulder	40	FFA, C_{31},C_{33},C_{35} K
DBC 8*	A10	Carinated bowl	Shoulder	108	DAF, C_{31},C_{33},C_{35} K
DBC 9*	A10	Carinated bowl	Rim	301	DAF
DBC 10	A10	Carinated bowl	Rim	0	
DBC 11*	A10	Carinated bowl	Rim	565	DAF, C_{31},C_{33},C_{35} K, WE
DBC 12*	A10	Plain bowl	Rim	60	DAF, WE, OH
DBC 13A*	A10	Carinated bowl	Rim	1067	DAF, C_{31},C_{33},C_{35} K
DBC 13B	A10	Carinated bowl	Rim	44	DAF, WE, AL
DBC 14	A10	Carinated bowl	Rim	0	
Following sherds are either plain or carinated bowls					
DBC 16	A6		Rim	156	DAF, C_{31},C_{33},C_{35} K, degraded beeswax
DBC 17	A6		Rim	0	
DBC 18	A6		Rim	0	
DBC 19	A6		Rim	48	DAF, WE, AL
DBC 20*	A6		Rim	44	DAF
DBC 21*	A6		Rim	251	DAF, C_{31},C_{33},C_{35} K
DBC 22*	A6		Rim	735	DAF, C_{31},C_{33},C_{35} K
DBC 23	A6	Fengate ware	Rim	10	C_{31},C_{33},C_{35} K
DBC 24	A6		Rim	0	
DBC 25*	A6		Rim	1033	DAF
DBC 26	A6		Rim	0	
DBC 27	A6		Rim	0	
DBC 28*	A6		Rim	334	DAF

* Extracts analysed by GC-C-IRMS

Table 10. Details of Grooved Ware (Early Neolithic) and Peterborough Ware (Late Neolithic) from the Upper Ninepence excavation (Walton U9D 94) and descriptions of absorbed (AR) and carbonised (CR) residues analysed.

Sherd no.		Feature (Context no.)	Decoration	Lipid Content ($\mu g\ g^{-1}$)	Lipid components present
Peterborough Ware (*ca* 3000 BC)					
P1a*	AR	Pit 13 (3)	Twisted cord impressions as multiple horizontal lines.	120	FFA, C_{31},C_{33},C_{35} K
P1b*	AR	Pit 13 (3)	Incised decoration in diagonal lines on inside of vessel	104	DAF, C_{31},C_{33},C_{35} K
P1	CR	Pit 13 (3)		Trace	-
P3*	AR	Mound (2)	Incised internal crosshatching. Birdbone impressions on top of rim	338	DAF
P5*	AR	Pit 16 (17)	Oblique fingernail impressions	210	FFA, C_{31},C_{33},C_{35} K
P10	AR	Pit 6 (7)	Random fingernail impressions	13	-
Grooved Ware (*ca* 2500 BC)					
P21	AR	Pit 198 (289)		Trace	-
P21	CR	Pit 198 (289)		Trace	-
P28	AR	Pit 198 (289)	Double cordon with fingernail impressions	0	-
P28	CR	Pit 198 (289)		0	-
P33	AR	Pit 154 (155)		13	-
P33*	CR	Pit 154 (155)	Large barrel-shaped vessel. Well-defined horizontal and vertical cordons. Largely undecorated	434	DAF
P34	AR	Pit 154 (155)	Internal and upper part of outer surface decorated with incised triangular motifs	0	-
P37	AR	Pit 198 (199/289) Pit 198 (191)			
P38	AR	Pit 154 (155)		12	DAF
P38*	CR	Pit 154 (155)		314	DAF
P39	AR	Pit 154 (155)	Similar to P33	7	DAF
P39*	CR	Pit 154 (155)		669	DAF
P48	AR	Pit 198 (199/289)		Trace	-
P62	AR	(87)		Trace	-

Table 10. Continued.

P65	AR	(294)		Trace	-
P66*	AR	(133)		118	DAF
P68*	AR	(133)	Internally decorated vessel.	247	DAF
P68	CR	(133)	Interior has incised motifs-triangular with herringbone or oblique infill	279	DAF

* Extracts analysed by GC-C-IRMS

Table 11. Lipid content and description of lipid components in ethnographic vessels.

Sample	Fat/oil processed	Lipid content ($\mu g\ g^{-1}$)	Description of lipid components
Vessel A	Pork	6493	DAF, including $C_{17:0}$ fatty acid and C_{18} dihydroxyacid.
Vessel B	Dairy	55	$C_{14:0}$, $C_{15:0}$, $C_{16:0}$, $C_{17:0}$, C_{17}br, $C_{18:0}$ and $C_{18:1}$ free fatty acids.
Vessel C	Olive oil	3576	C_7, C_8 and C_9 diacids, $C_{16:0}$, $C_{18:0}$, $C_{18:1}$ and $C_{20:0}$ free fatty acids ($C_{18:1}$ predominating), C_{18} dihydroxyacids, minor DAG and TAG.
Vessel F	Pork	10832	C_8 and C_9 diacids, $C_{14:0}$, $C_{16:0}$, $C_{18:0}$ and $C_{18:1}$ free fatty acids, C_{16} and C_{18} dihydroxyacids.
Vessel G	Dairy	7	Free fatty acids ranging from C_8 to C_{18}, including C_{15} and C_{17} branched and straight-chain and $C_{18:1}$ and C_{16} and C_{18} 10-hydroxy acids, the latter particularly abundant.

APPENDIX 2

APPENDIX 2. PUBLISHED DATA ON FATTY ACID COMPOSITIONS OF MODERN ANIMAL FATS AND FISH OILS.

Table 1. Fatty acid composition of cow depot fats.

Sample	Reference	$C_{14:0}$	$C_{16:0}$	$C_{17:0}$	$C_{18:0}$	$C_{18:1}$ (total)
Beef tallow; range (% wt)	Spencer *et al.* (1976)	1.4-6.3	20-37	0.5-2.0	6-40	26-50
Beef back fat; rump; mean of bottom and top layers	Chacko and Perkins (1965)	6.05	33.4	nd	11.75	40.7
Beef back fat; perinephric	Chacko and Perkins (1965)	5.2	35.3	nd	20.3	36.2
Beef back fat; visceral pericardial	Chacko and Perkins (1965)	2.1	26.9	nd	34.2	29.0
Beef back fat; ribs	Chacko and Perkins (1965)	4.4	32.5	nd	15.7	39.9
Beef brisket; mean of 3 (%m/m)	Whitehead (1988)	3.17	22.4		8.13	46.6
Beef cod; mean of 3 (%m/m)	Whitehead (1988)	4.03	27.37		18.3	36.7
Beef flank; mean of 3 (%m/m)	Whitehead (1988)	3.63	25.7		16.23	40.5
Beef suet; mean of 3 (%m/m)	Whitehead (1988)	3.5	27.2		25.0	31.9
Beef (%m/m)	Saeed, Abu-Dagga and Rhaman (1986)	2.9	32.5	1.2	23.8	31.3
Beef; grain fed adipose (% normalised)	Marmer *et al.* (1984)	3.28	25.99	1.99	12.5	46.48
Beef; forage fed adipose (% normalised)	Marmer *et al.* (1984)	3.92	27.07	1.09	15.6	38.39

[1] nd=not detected

Table 2 . Fatty acid composition of dairy fats.

Sample	Reference	$C_{14:0}$	$C_{16:0}$	$C_{17:0}$br	$C_{18:0}$	$C_{18:1}$ (total)
Milk fats in June; mean 4-8 samples	Jensen *et al.* (1962)	9.01	22.05	nd	14.27	30.41
Milk fats in December; mean 4-8 samples;	Jensen *et al.* (1962)	10.58	25.98	nd	11.58	24.75
Butter oil (% of total FAME)	Iverson *et al.* (1965)	11.2	27.8	0.59	12.1	30.3
Cow milk (wt%)	Glass *et al.* (1967)	9.5	26.3	0.5	14.6	29.8
Sheep milk (wt%)	Glass *et al.* (1967)	11.8	25.4	2.0	9.0	20.0
Goat milk(wt%)	Glass *et al.* (1967)	10.3	24.6	0.7	12.5	28.5

Table 3. Fatty acid composition of sheep fats.

Sample	Reference	$C_{14:0}$	$C_{16:0}$	$C_{17:0}$br	$C_{18:0}$	$C_{18:1}$ (total)
Lard (wt%)	Iverson *et al.* (1965)	1.27	24	nd[1]	13	43.5
Lard; range (% wt)	Spencer *et al.* (1976)	0.5-2.5	20-32	0.5	5.0-24	35-62
Chest; mean 4-6; 4-5yrs (wt%±SEM)	Duncan and Garton (1967)	2.3±0.18	26.2±1.2	1.5±0.09	20.7±1.84	42.6±3.21
Rump; mean 4-6; 4-5yrs (wt%±SEM)	Duncan and Garton (1967)	2.2±0.20	23.4±1.3	1.3±0.13	11.1±0.50	53.2±1.91
Perirenal; mean 4-6; 4-5yrs (wt%±SEM)	Duncan and Garton (1967)	2.4±0.22	25.6±1.2	1.4±0.04	33.9±1.20	29.5±1.49
Mesenteric; mean 4-6; 4-5yrs (wt%±SEM)	Duncan and Garton (1967)	2.0±0.21	24.2±1.3	1.6±0.08	33.3±1.79	32.5±2.02
Lamb breast; mean of 3 (%m/m)	Whitehead and Turrell (1988)	4.38	20.6		19.2	40.63
Lamb body; mean of 3 (%m/m)	Whitehead and Turrell (1988)	3.83	20.8		25.0	36.47
Lamb shoulder; mean of 3 (%m/m)	Whitehead and Turrell (1988)	3.53	20.3		25.0	37.47
Subcutaneous fat; wether lambs; mean 32 (% wt)	Busboom *et al.* (1981)	1.7	15.2		9.0	34.9
Subcutaneous fat; ram lambs; mean 32 (% wt)	Busboom *et al.* (1981)	1.7	13.0		5.2	32.8
Perirenal (mol%)	Christie and Moore (1971)	2.5	21.4	1.4	34.7	31.4
Omental (mol%)	Christie and Moore (1971)	2.2	23.0	1.5	31.9	34.1
Subcutaneous; chest (mol%)	Christie and Moore (1971)	2.6	23.0	1.9	25.8	37.9
New-born lamb perirenal (mol%)	Noble *et al.* (1971)	-	21.0	-	14.1	62.1
Sheep total fatty tissues; pasture-fed	Brooker and Shorland (1950)	3.5	25.0		22.2	44.2
Lamb (%m/m)	Saeed *et al.* (1986)	6.9	21.3	1.8	6.5	37.3

Table 4. Fatty acid composition of goat fat.

Sample	Reference	$C_{14:0}$	$C_{16:0}$	$C_{17:0}$br	$C_{18:0}$	$C_{18:1}$ (total)
Goat (wt%)	Garton and Duncan (1971)	2.1	25.5	nd	28.1	38.4

Table 5. Fatty acid composition of deer fats.

Sample	Reference	$C_{14:0}$	$C_{16:0}$	$C_{17:0}$br	$C_{18:0}$	$C_{18:1}$ (total)
Deer perinephric; pasture-fed	Shorland (1953)	5.1	35.9		29.6	17.0
Red deer (wt%)	Garton and Duncan (1971)	5.7	30.9	nd	35.7	15.4

Table 6. Fatty acid composition of pig fats.

Sample	Reference	$C_{14:0}$	$C_{16:0}$	$C_{17:0}$	$C_{18:0}$	$C_{18:1}$ (total)
Pig back fat; rump; mean of bottom and top layers	Chacko and Perkins (1965)	1.75	30.4		15.8	35
Pig back fat; shoulder; mean of bottom and top layers	Chacko and Perkins (1965)	1.8	25.15		13.45	86.3
Pig perinephric	Chacko and Perkins, (1965)	1.7	30.6		11.3	41.8
Pig flank	Chacko and Perkins (1965)	2.0	24.3		16.9	38.0
Pork backfat; mean of 3 (%m/m)	Whitehead (1988)	1.4	23.9		11.7	45.2
Pork belly; mean of 3 (%m/m)	Whitehead (1988)	1.6	26.8		15.2	38.8
Pork flare; mean of 3 (%m/m)	Whitehead (1988)	1.6	28.8		31.3	32.6
Pork head; mean of 3 (%m/m)	Whitehead (1988)	1.4	24.3		12.6	42.7
Pork (%m/m)	Saeed, Abu-Dagga and Rhaman (1986)	1.6	25.5	0..3	12.8	45.7

Table 7. Fatty acid composition of fat from pigs fed milk or whey as a supplement to their diet.

Sample	Reference	$C_{14:0}$	$C_{16:0}$	$C_{17:0}$br	$C_{18:0}$	$C_{18:1}$ (total)
Separated milk with addition of mixed meal (high plane of nutrition; %m/m)	Hilditch *et al.* (1939)	nd	35.9	nd	20.1	40.6
Separated milk with addition of mixed meal (low plane of nutrition; %m/m)	Hilditch *et al.* (1939)	nd	33.3	nd	21.0	44.6
Skim milk only (%m/m)	Shorland and de la Mare (1945)	nd	29.5	nd	10.9	52.9
Skim milk and maize meal (%m/m)	Shorland and de la Mare (1945)	nd	34.7	nd	14.4	47.6

Table 8. Fatty acid composition of horse fats.

Sample	Reference	$C_{14:0}$	$C_{16:0}$	$C_{17:0}$br	$C_{18:0}$	$C_{18:1}$ (total)
Horse total fatty tissues; pasture-fed	Shorland and Bruce (1952)	2.4	29.7		4.3	32.5
Horse (wt%)	Garton and Duncan (1971)	2.0	26.7	nd	6.2	32.8

Table 9. Fatty acid composition of rabbit fats.

Sample	Reference	$C_{14:0}$	$C_{16:0}$	$C_{17:0}$br	$C_{18:0}$	$C_{18:1}$ (total)
Wild rabbit; abdominal; pasture-fed	Shorland (1953)	1.6	22.1		6.4	12.7

Table 10. Fatty acid composition of fish oils.

Sample	***Reference***	$C_{14:0}$	$C_{16:0}$	$C_{17:0}$	$C_{18:0}$	$C_{18:1}$ **(total)**
Menhaden range (high PUFA; %m/m)	Whitehead and Turrel (1988)	5.5-10.2	17.1-19.8	0.8	3.2-7.8	12.1-18.8
Sardine (high PUFA; %m/m)	Whitehead and Turrel (1988)	7.7	17.8		2.3	11.1
Pilchard (high PUFA; %m/m)	Whitehead and Turrel (1988)	6.6	16.6	0.6	3.4	10.4
Herring range (high PUFA; %m/m)	Whitehead and Turrel (1988)	4.8-8.0	10.5-14.4	0.3	0.7-2.5	10.0-13.3
Pout and sprat (high MUFA; %m/m)	*Whitehead and Turrel (1988)*	5.5	14.8	0.5	1.9	13.9
Capelin	Ackman (1980)	7.85	8.81	0.06	0.72	8.45
Mackerel	Ackman (1980)	7.81	15.93	0.20	1.73	12.93
Atlantic Herring	Ackman (1980)	8.77	14.84	0.04	0.97	14.57
Cod liver oil	Addison *et al.* (1968)	4.9	12.4		1.8	22.6 (n-9)

APPENDIX 3

APPENDIX 3. FAME DISTRIBUTIONS IN MODERN AND ARCHAEOLOGICAL FATS AND OILS.

Table 1. Free fatty acid distributions[1] (%) in subcutaneous fats[2] and kidney[3] and subcutaneous back[4] fat from sheep.

	Fatty acid									
Sample	**$C_{12:0}$**	**$C_{14:0}$**	**C_{15}br**	**$C_{15:0}$**	**$C_{16:0}$**	**$C_{16:1}$**	**C_{17}br**	**$C_{17:0}$**	**$C_{18:0}$**	**$C_{18:1}$**
Ewe[2]	nd[5]	1.7	0.7	0.7	20.8	0.9	1.7	2.2	32.9	38.4
Heb lamb[2]	0.3	5.2	0.5	0.6	25.1	0.3	2.0	1.2	21.9	42.8
Mutton[2]	nd	1.4	0.3	0.3	19.3	0.6	0.5	1.9	36.7	39.1
Mutton leg[2]	nd	1.6	0.5	0.5	18.0	1.2	1.4	1.8	29.4	45.7
Mutton shoulder[2]	nd	1.9	0.4	0.5	20.1	1.9	1.6	1.5	24.0	48.1
Ram lamb 1[2]	nd	2.5	nd	1.0	35.9	nd	4.8	4.4	39.6	11.8
Ram lamb 2[2]	nd	2.9	0.6	0.6	20.0	2.2	1.6	1.7	26.2	44.3
L1K91[3]	nd	2.2	0.6	0.5	17.1	0.9	1.3	1.7	53.3	22.3
L1B91[4]	0.1	3.5	0.6	0.7	22.2	1.8	1.5	1.6	29.8	38.2
L2K91[3]	1.3	3.4	0.9	0.9	21.0	1.8	1.6	2.1	39.0	28.0
L2B91[4]	0.2	4.6	0.9	1.0	24.8	2.2	1.5	2.2	28.8	33.8
L3K91[3]	0.2	3.0	1.0	0.9	21.1	1.3	1.6	2.0	44.6	24.4
L3B91[4]	0.2	3.4	0.9	0.9	23.4	1.6	1.6	2.1	33.3	32.7
Mean	0.2	2.9	0.6	0.7	22.2	1.3	1.7	2.0	33.8	34.6

[1] Fats were saponified and the free acids converted to their methyl ester derivatives prior to analysis by GC.

[5] nd=none detected.

Table 2. Free fatty acid distributions (%) in subcutaneous brisket[1], rump[2] and kidney[3] fats from cows.

	Fatty acid											
Sample	**$C_{12:0}$**	**$C_{14:0}$**	**C_{15}br**	**$C_{15:0}$**	**$C_{16:0}$**	**$C_{16:1}$**	**C_{17}br**	**$C_{17:0}$**	**$C_{18:0}$**	**$C_{18:1}$**	**$C_{18:2}$**	**$C_{18:3}$**
C1BB[1]	nd	3.8	0.6	0.8	28.9	3.0	1.3	1.5	21.5	37.5	0.5	0.7
C1BR[2]	nd	3.5	0.7	0.9	28.8	1.5	1.3	1.9	30.7	29.6	0.5	0.6
C1BK[3]	nd	3.2	0.7	0.8	26.1	6.3	1.5	1.3	12.5	46.4	0.5	0.8
C2BB[1]	nd	4.5	0.7	0.5	27.3	4.7	1.3	1.2	16.1	42.1	1.2	0.4
Mean		3.8	0.7	0.7	27.8	3.9	1.4	1.5	20.2	38.9	0.7	0.6

Table 3. Free fatty acid distributions (%) in subcutaneous brisket[1], rump[2] and kidney[3] fats from deer.

	Fatty acid											
Sample	$C_{12:0}$	$C_{14:0}$	C_{15}br	$C_{15:0}$	$C_{16:0}$	$C_{16:1}$	C_{17}br	$C_{17:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
D1BB[1]	nd	2.2	nd	1.0	23.3	4.5	2.0	2.7	8.5	54.7	nd	1.1
D1BR[2]	nd	3.0	0.4	0.6	20.5	3.7	1.4	1.4	15.8	51.5	0.7	1.1
D1BK[3]	nd	1.9	0.5	0.4	19.0	1.2	1.3	1.6	35.5	37.2	0.6	0.9
D2BB[1]	nd	5.3	1.2	1.1	33.2	4.2	1.3	1.3	29.2	23.4	nd	nd
D2BR[2]	0.2	9.5	0.5	0.5	30.8	24.4	0.7	0.4	5.3	27.0	0.3	0.6
D3BR[1]	nd	2.8	1.2	1.1	26.0	1.7	1.1	1.2	41.6	20.7	0.9	1.6
D3BK[3]	nd	2.0	1.2	1.1	23.7	1.3	1.2	1.4	51.7	16.5	nd	nd
Mean	0.03	3.8	0.7	0.8	25.2	5.9	1.3	1.4	26.8	33.0	0.4	0.8

Table 4. Free fatty acid distributions (%) in cows milk fats.

	Fatty acid						
Sample	$C_{10:0}$	$C_{12:0}$	$C_{14:0}$	C_{15}br	$C_{15:0}$	$C_{16:0}$	$C_{16:1}$
Sarah 1 (prebirth)	0.2	1.6	12.5	0.8	1.2	47.0	2.5
Sarah 2 (Colostrum-post calving)	0.3	1.8	11.6	0.6	1.1	41.1	1.9
Mallard 1	0.2	0.8	6.5	1.2	1.6	35.8	2.4
Mallard 2 (2 weeks post calving)	0.4	1.9	8.8	1.3	1.3	23.9	1.4
Twinkle (early Feb 1998)	0.2	2.5	14.2	0.5	0.8	40.7	2.9
Tulip 1 (colostrum)	0.1	1.0	7.8	0.2	0.9	29.8	2.7
Tulip 2 (2-3 days post calving)	0.3	2.4	12.9	0.6	1.2	36.4	1.5
Twinkle 2 (25th Feb 1998)	0.5	2.4	12.2	1.2	1.6	34.8	3.0
Mean	0.3	1.8	10.8	0.8	1.2	36.2	2.3

Table 4. Continued.

	Fatty acid					
Sample	C_{17}br	$C_{17:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
Sarah 1 (prebirth)	1.1	1.1	11.2	18.7	1.2	1.1
Sarah 2 (Colostrum-post calving)	1.1	1.0	13.2	24.1	1.1	1.0
Mallard 1	1.9	2.1	14.8	31.7	0.5	0.5
Mallard 2 (2 weeks post calving)	1.5	0.9	35.6	20.4	1.4	1.2
Twinkle (early Feb 1998)	0.9	0.9	10.4	22.8	2.1	1.1
Tulip 1 (colostrum)	1.2	1.2	19.5	32.2	2.3	1.1
Tulip 2 (2-3 days post calving)	0.9	1.0	16.7	23.5	1.5	1.1
Twinkle 2 (25th Feb 1998)	1.6	1.4	10.5	28.7	1.0	1.0
Mean	1.3	1.2	16.5	25.3	1.4	1.0

Table 5. Free fatty acid distributions (%) in sheep milk fats.

	Fatty acid						
Sample	$C_{10:0}$	$C_{12:0}$	$C_{14:0}$	C_{15}br	$C_{15:0}$	$C_{16:0}$	$C_{16:1}$
Heb lamb 1	trace	4.7	13.7	1.1	2.0	30.4	1.8
Heb lamb 2	2.2	2.4	10.2	1.2	1.4	26.0	2.7
Mean	1.1	3.5	11.9	1.2	1.7	28.2	2.3

Table 5. Continued.

	Fatty acid					
Sample	C_{17}br	$C_{17:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
Heb lamb 1	2.1	2.1	25.4	11.8	2.7	2.1
Heb lamb 2	1.2	1.0	26.4	22.9	0.7	1.8
Mean	1.7	1.6	25.9	17.3	1.7	1.9

Table 6. Free fatty acid distributions (%) in subcutaneous tail fats from pigs.

	Fatty acid							
Sample	$C_{14:0}$	$C_{16:0}$	$C_{16:1}$	$C_{17:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
P1T121	1.2	24.0	3.1	0.5	12.1	43.3	14.3	1.5
P2T121	1.4	24.3	0.5	0.3	9.9	43.7	18.1	1.9
P3T121	1.4	25.0	3.3	0.2	10.5	44.7	14.5	0.4
P4T121	1.5	26.1	3.6	0.4	12.3	43.8	11.5	1.0
P5T121	1.2	20.1	3.1	0.4	10.4	43.8	19.2	1.8
P6T121	1.4	24.8	3.5	0.5	11.9	42.8	13.8	1.3
P7T121	1.4	22.4	3.7	0.4	8.9	44.3	17.3	1.6
P8T121	1.4	23.1	3.8	0.3	10.0	46.2	14.1	1.2
C_3 pig	1.1	27.3	2.0	0.4	17.2	45.6	5.3	1.1
Mean	1.3	24.1	2.9	0.4	11.5	44.2	14.2	1.3

Table 7. Free fatty acid distributions (%) in horse subcutaneous thigh[1] and internal peritoneal[2] fat

	Fatty acid										
Sample	$C_{12:0}$	$C_{14:0}$	$C_{15:0}$	$C_{16:0}$	$C_{16:1}$	C_{17}br	$C_{17:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
H1PL[1]	nd	3.2	0.3	24.8	4.5	0.3	0.6	6.4	30.7	8.0	21.3
H1PP[2]	nd	3.0	0.4	29.2	6.1	0.2	0.6	4.6	26.4	7.6	22.1
H3PL	nd	3.0	0.3	22.9	4.9	0.3	0.6	6.6	34.7	6.4	20.5
H3PP	0.1	3.3	0.3	22.2	3.6	0.2	0.6	7.1	31.8	6.8	24.0
H4PL	nd	4.0	0.3	24.3	5.9	0.2	0.5	5.9	29.2	7.0	22.8
H4PP	nd	3.4	0.3	24.4	4.2	0.2	0.6	7.1	28.8	6.9	24.1
H5PL	nd	3.1	0.3	24.1	3.8	0.1	0.7	7.2	27.8	9.5	23.4
H5PP	nd	3.2	0.3	23.9	3.1	0.1	0.7	8.7	27.6	8.3	24.2
H6PL	nd	4.7	0.3	27.2	6.5	0.2	0.4	4.8	32.0	8.4	15.5
H6PP	nd	4.2	0.4	26.3	6.6	0.2	0.5	4.1	32.5	10.0	15.4
Mean	0.01	3.5	0.3	24.9	4.9	0.6	0.2	6.2	30.2	7.9	21.3

Table 8. Free fatty acid distributions (%) in subcutaneous chicken fat.

	Fatty acid						
Sample	$C_{14:0}$	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
CHICK5	0.7	29.9	5.6	9.6	44.7	9.2	0.3
CHICK6	0.3	24.4	8.1	5.5	45.2	15.4	1.1
CHICK8	0.1	23.2	6.9	4.7	41.3	21.4	2.3
Mean	0.4	25.8	6.9	6.6	43.8	15.3	1.2

Table 9. Free fatty acid distributions (%) in subcutaneous goose fat.

	Fatty acid							
Sample	$C_{14:0}$	$C_{16:0}$	$C_{16:1}$	$C_{17:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
Goose 1	0.5	28.2	4.5	nd	6.1	60.7	nd	nd
Goose 2	0.4	23.8	4.6	nd	5.1	59.2	6.1	0.9
Goose 3	0.3	21.8	3.2	nd	7.0	61.7	5.4	0.6
Goose 4	1.8	21.4	3.7	0.5	6.1	58.8	6.3	1.3
Mean	0.8	23.8	4.0	0.1	6.1	60.1	4.5	0.7

Table 10. Free fatty acid distributions (%) in fish oil.

	Fatty acid								
Sample	$C_{14:0}$	$C_{15:0}$	$C_{16:0}$	$C_{16:1}$	$C_{17:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{20:1}$
Cod	1.4	nd	50.3	1.5	nd	15.4	25.5	1.3	4.6
Haddock	1.9	0.7	50.3	3.8	0.7	11.7	25.1	2.0	3.9
Plaice	4.4	0.6	43.9	8.3	nd	11.4	21.7	2.7	7.0
Mean	2.6	0.5	48.2	4.5	0.23	12.8	24.1	2.0	5.1

Table 11. Free fatty acid distributions (%) in olive oil.

	Fatty acid						
Sample	$C_{16:0}$	$C_{16:1}$	C_{17}br	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
Olive oil	5.1	0.4	0.1	2.5	87.1	4.7	0.1

Table 12. Free fatty acid distributions[1] (%) in remnant fats from West Cotton, Northamptonshire (Late Saxon/early medieval).

	Fatty acid				
Sherd no.	$C_{14:0}$	$C_{16:0}$	$C_{17:0}$ br	$C_{17:0}$	$C_{18:0}$
RP2	3.5	50.7	1.2	1.3	43.3
RP4	1.2	49.2	0.0	1.2	48.5
RP6	3.7	43.7	1.2	2.9	48.6
RP7	2.6	34.2	1.3	2.4	59.5
RP10	3.1	44.8	nd	1.7	50.4
RP13	1.7	34.4	1.4	3.1	59.4
RP16	5.9	37.2	1.8	2.6	52.5
RP22	8.6	49.0	2.4	2.0	38.0
RP28	3.5	47.0	1.2	1.9	46.4
RP30	8.5	43.1	2.0	2.4	44.0
RP50	1.6	31.3	1.0	2.5	63.7
RP53	1.9	32.1	1.3	2.6	62.2
RP58	3.5	36.4	1.9	2.6	55.6
RP60	6.3	42.5	1.9	2.6	46.7
RP61	2.8	35.1	1.4	2.1	58.7
RP71	2.2	29.3	1.9	2.9	63.8
RP72	5.5	40.8	1.9	2.6	49.3
RP73	4.2	46.5	0.9	1.8	46.6
RP78	3.6	44.0	1.6	2.5	48.3
RP81	3.2	32.6	1.6	3.2	59.5
RP82	2.7	38.9	1.1	2.5	54.8
RP83	2.5	41.0	1.1	2.5	52.9
RP85	2.1	38.1	1.5	2.8	55.5
RP86	2.3	36.5	0.9	2.1	58.2
RP87	1.3	31.6	1.0	2.5	63.6
RP88	1.7	45.2	nd	1.3	51.9
RP89	3.9	40.4	2.0	3.8	49.8
RP91	9.7	50.1	1.7	2.4	36.0
RP93	5.5	33.6	1.6	2.7	56.6
RP94	3.0	34.2	1.0	2.1	59.8

[1] Remnant fats were saponified and converted to their methyl ester derivatives prior to analysis by GC.

Table 13. Free fatty acid distributions (%) in remnant fats from Stanwick, Northamptonshire (Iron Age/Romano-British).

	Fatty acid				
Sherd no.	$C_{14:0}$	$C_{16:0}$	$C_{17:0}$ br	$C_{17:0}$	$C_{18:0}$
ST 152	2.3	32.8	1.0	2.8	61.1
ST 153	2.9	34.7	1.3	4.1	57.1
ST 156	2.6	31.3	1.3	3.1	61.8
ST 160	5.5	39.5	1.6	3.5	50.0
ST 161	8.6	48.1	1.7	2.4	39.2
ST 190 BOD	2.3	40.7	1.5	3.0	52.4
ST 190	4.6	42.9	1.6	2.8	48.2
ST 193	4.4	34.3	1.2	2.3	57.8
ST 194	6.6	54.8	1.7	2.4	34.6
ST 197	3.0	46.2	1.7	2.8	46.3
ST 206 RIM	2.9	33.9	2.0	3.4	57.8
ST 206 BOD	6.9	39.4	1.4	2.6	49.8
ST 208	14.3	47.0	2.4	2.2	34.1
ST 210	4.5	43.8	1.7	3.1	47.0
ST 211	1.0	31.5	1.9	3.8	61.9
ST 212	3.6	42.0	2.9	3.1	48.4
ST 215	1.9	30.6	1.8	3.6	62.2
ST 226	2.5	21.3	1.0	3.4	72.0
ST 262	3.9	38.8	1.3	2.7	53.3

Table 14. Free fatty acid distributions (%) in remnant fats from Wickham Bonhunt, Essex (Romano-British and Middle Saxon).

	Fatty acid				
Sherd no.	$C_{14:0}$	$C_{16:0}$	$C_{17:0}$ br	$C_{17:0}$	$C_{18:0}$
1	2.3	44.6	1.2	3.1	48.9
2	2.9	39.2	1.3	2.6	54.0
3	1.9	37.8	0.9	1.9	57.5
6	2.5	44.9	1.2	2.3	49.2
7	3.1	43.5	1.4	2.8	49.3
8	2.2	47.6	0.7	1.6	47.9
10	4.3	43.1	1.8	3.7	47.1
12	1.4	33.3	1.0	2.8	61.5
13	2.0	33.7	1.7	3.3	59.3
15	3.6	44.7	1.6	2.3	47.8
16	1.8	54.1	0.7	1.4	42.1
19	3.0	36.0	1.3	3.0	56.6

Table 15. Free fatty acid distributions (%) in remnant fats from Yarnton Cresswell field (Early-Middle Iron Age).

	Fatty acid				
Sherd no.	$C_{14:0}$	$C_{16:0}$	$C_{17:0}$ br	$C_{17:0}$	$C_{18:0}$
101	4.4	32.1	1.1	2.9	59.6
104	nd	79.3	nd	nd	20.7
108	nd	28.8	nd	nd	71.2
112	2.3	49.4	1.4	5.7	41.2
113	1.4	30.0	1.1	2.3	65.1
114	nd	58.5	nd	nd	41.5
117	nd	28.6	nd	nd	71.4
118	2.9	28.4	1.3	2.5	64.9
119	12.3	30.3	1.1	2.5	53.8
121	9.6	52.2	1.7	2.1	34.4
126	4.5	40.9	1.7	2.7	50.2
127	5.6	46.9	3.4	2.2	41.8
128	6.8	56.2	2.2	2.5	32.3
129	0.4	55.1	1.6	2.0	41.0
130	10.9	41.5	1.3	2.2	44.1
135	nd	44.4	nd	nd	55.6
136	5.7	51.7	nd	nd	42.6
137	8.0	47.0	1.9	2.0	41.1
141	8.8	43.8	1.4	1.9	44.2
142	nd	41.9	nd	nd	58.1
146	6.3	44.9	1.7	1.9	45.2
147	6.6	41.3	3.1	3.7	45.3
148	9.4	39.5	1.4	2.4	47.3
149	7.8	29.7	1.9	3.0	57.6
144	1.8	46.0	0.8	1.9	49.6

Table 16. Free fatty acid distributions (%) in remnant fats from Yarnton flood plain (Neolithic-Bronze Age).

	Fatty acid				
Sherd no.	**$C_{14:0}$**	**$C_{16:0}$**	**$C_{17:0}$ br**	**$C_{17:0}$**	**$C_{18:0}$**
1	nd	35.4	nd	3.1	61.6
5	nd	45.3	nd	3.2	51.5
21	3.5	42.8	nd	2.5	51.3
23	13.7	47.9	nd	1.5	36.8
31	1.6	39.8	nd	2.8	55.8
32	nd	60.5	nd	nd	39.5
33	7.9	51.8	2.8	1.9	35.7
38	nd	47.3	nd	nd	52.7
40	nd	42.1	nd	nd	57.9
41	9.2	48.3	1.3	2.8	38.4
44	11.2	35.6	2.9	1.3	49.0
46b	nd	52.7	nd	nd	47.3
49	3.9	36.0	1.1	2.8	56.2
50	4.2	23.6	0.7	2.0	69.6

Table 17. Free fatty acid distributions (%) in remnant fats from Eton Lake End Road (Late Neolithic/Early Bronze Age).

	Fatty acid				
Small find no.	**$C_{14:0}$**	**$C_{16:0}$**	**$C_{17:0}$ br**	**$C_{17:0}$**	**$C_{18:0}$**
2054	1.6	50.4	1.2	2.5	44.4
2041-8	1.4	24.4	0.4	1.5	72.3
2041-11	1.4	29.2	0.5	2.0	66.9
2041-12	2.6	35.9	1.0	1.7	58.8
2041-rim	1.5	32.3	1.0	2.6	62.6
2271	0.4	43.1	0.6	1.8	54.2
2163	5.6	53.9	2.1	2.5	35.9
2164	0.4	43.8	1.0	2.0	52.8
2166	2.6	48.8	1.9	3.0	43.7
1135	nd	17.2	nd	1.6	81.2
865	5.1	50.2	1.6	2.4	40.8
571	nd	34.6	nd	nd	65.4
601	0.6	31.7	0.8	2.0	64.9
772	1.9	24.8	nd	3.6	69.8

Table 18. Free fatty acid distributions (%) in remnant fats from Eton Rowing Lake (Early Neolithic).

	Fatty acid				
Sherd no.	**$C_{14:0}$**	**$C_{16:0}$**	**$C_{17:0}$ br**	**$C_{17:0}$**	**$C_{18:0}$**
DBC1	1.6	30.8	1.6	2.9	63.2
DBC3	2.6	35.3	nd	2.4	59.8
DBC7	1.2	21.0	1.9	4.1	71.9
DBC8	1.2	20.4	0.5	4.2	73.8
DBC9	3.3	38.3	2.0	7.4	49.0
DBC11	0.8	29.7	0.9	3.0	65.5
DBC12	0.3	14.4	0.6	2.6	82.1
DBC13A	2.1	37.9	1.4	4.0	54.6
DBC13B	4.6	19.8	nd	nd	75.7
DBC16	1.9	37.8	1.3	2.4	56.6
DBC19	2.3	30.4	0.9	2.8	63.6
DBC20	4.5	42.9	2.0	2.2	48.4
DBC21	4.7	44.5	1.5	3.0	46.4
DBC22	6.4	47.8	1.4	3.0	41.4
DBC25	1.3	32.5	0.8	3.3	62.0
DBC28	5.8	49.9	1.5	2.7	40.1

Table 19. Free fatty acid distributions (%) in remnant fats from Walton, Mid Wales (Early-Late Neolithic).

	Fatty acid				
Sherd no.	**$C_{14:0}$**	**$C_{16:0}$**	**$C_{17:0}$ br**	**$C_{17:0}$**	**$C_{18:0}$**
P1a	0.7	23.6	1.3	7.5	66.9
P1b	2.6	30.5	0.9	2.7	63.4
P3	2.2	38.0	1.1	2.0	56.7
P5	0.2	9.9	1.0	5.0	83.9
P33 res	2.9	33.9	1.9	5.2	56.1
P38res	4.7	35.2	2.3	4.5	53.4
P39 res	6.7	43.4	2.3	4.0	43.6
P66	0.1	32.9	0.7	3.5	62.9
P68	0.6	37.8	0.7	3.2	57.8

Table 20. Free fatty acid distributions (%) in lipid residues from the Ethnographic vessels.

	Fatty acid				
Sample no.	**$C_{8:0}$**	**$C_{10:0}$**	**$C_{12:0}$ br**	**$C_{14:0}$**	**$C_{15:0}$br**
Vessel A	nd	nd	0.6	3.9	nd
Vessel B	nd	nd	nd	7.2	1.0
Vessel C	nd	nd	nd	nd	nd
Vessel F	nd	nd	nd	5.7	nd
Vessel G	0.5	1.0	3.4	15.6	1.4

Table 20. Continued.

	Fatty acid				
Sample no.	**$C_{15:0}$**	**$C_{16:0}$**	**$C_{17:0}$ br**	**$C_{17:0}$**	**$C_{18:0}$**
Vessel A	0.4	60.0	0.4	1.2	33.6
Vessel B	2.0	55.0	1.6	1.9	31.4
Vessel C	nd	75.1	nd	nd	24.9
Vessel F	nd	71.8	nd	0.8	21.8
Vessel G	2.3	54.8	1.6	1.6	18.0

Table 21. Free fatty acid distributions (%) in remnant fats from Botai, Kazakhstan (Early Neolithic).

	Fatty acid				
Sherd no.	**$C_{14:0}$**	**$C_{16:0}$**	**$C_{17:0}$ br**	**$C_{17:0}$**	**$C_{18:0}$**
iicr	3.7	53.7	trace	3.2	39.4
iiicr	3.2	49.3	trace	3.1	44.4
ipot	3.7	74.1	trace	5.0	17.2
n26pot	3.1	58.0	trace	3.0	35.9
xviiipot	4.8	51.4	trace	2.5	41.3
n21pot	7.4	66.2	trace	5.1	21.3

Table 22. Free fatty acid distributions (%) in lipid extracts of the Siberian horse fats from the Ukok plateau.

	Fatty acid						
Sample	**$C_{14:0}$**	**$C_{16:0}$**	**$C_{17:0}$br**	**$C_{17:0}$**	**$C_{18:0}$**	**$C_{18:1}$**	**$C_{18:2}$**
CHEST H1	2.7	49.6	1.0	2.3	35.7	6.8	1.8
SKIN H1	7.5	64.3	0.3	1.6	19.1	5.4	1.9
SACRUM H2	11.1	50.0	1.4	1.0	16.3	15.9	4.3

Table 23. Ratios of abundance of the straight-chain $C_{14:0}$ and $C_{17:0}$ fatty acid components in reference fats and oils.

Sample	$C_{14:0}/C_{17:0}$ fatty acid ratio	Mean
Cows adipose		
C1BB	2.6	
C1BK	1.9	2.7
C1BR	2.5	
C2BB	3.7	
Cows milk		
Sarah 1 (prebirth)	11.6	
Sarah 2 (colostrum, post-calving)	11.5	
Mallard 1	3.1	
Mallard 2 (2 wks post calving)	9.4	9.9
Twinkle (early Feb '98)	15.4	
Tulip 1 (colostrum)	6.3	
Tulip 2 (2-3 days post birth)	13.2	
Twinkle 2 (25 Feb '98)	8.6	
Sheep adipose		
L1K91	1.3	
L1B91	2.2	
L2K91	1.6	
L2B91	2.1	
L3K91	1.5	
L3B91	1.6	1.6
Ewe	0.8	
Mutton (unspecified)	0.8	
Hebridean lamb	4.3	
Mutton (leg fat)	0.9	
Mutton (shoulder fat)	1.2	
Ram lamb 1 (10 months)	0.6	
Ram lamb 2 (10 months)	1.7	
Sheep milk		
Heb lamb 1 milk	10.4	8.4
Heb lamb 2 milk	6.3	
Deer fat		
D1BR	0.8	
D1BB	2.2	
D1BK	1.2	5.4
D2BR	4.2	
D2BB	25.6	
D3BR	2.3	
D3BK	1.4	
Horse adipose		
H1PL	5.2	

Table 23. Continued.

Sample	$C_{14:0}/C_{17:0}$ fatty acid ratio	Mean
H1PP	5.3	6.3
H3PL	5.2	
H3PP	5.3	
H4PL	7.7	
H4PP	6.0	
H5PL	4.6	
H5PP	4.4	
H6PL	10.6	
H6PP	9.1	
Pig adipose		4.0
Pig 1	2.5	
Pig 2	5.3	
Pig 3	7.2	
Pig 4	4.1	
Pig 5	3.0	
Pig 6	2.8	
Pig 7	3.4	
Pig 8	4.7	
C_3 pig	2.7	
Poultry fat		
Chicken 5	No $C_{17:0}$, $C_{14:0} < 1\%$	
Chicken 6	No $C_{17:0}$, $C_{14:0} < 1\%$	
Chicken 8	No $C_{17:0}$, $C_{14:0} < 1\%$	
Goose 1	No $C_{17:0}$, $C_{14:0} < 2\%$	
Goose 2	No $C_{17:0}$, $C_{14:0} < 2\%$	
Goose 3	No $C_{17:0}$, $C_{14:0} < 2\%$	
Goose 4	3.6	
Fish oil		
Cod	No $C_{17:0}$	
Haddock	2.7	
Plaice	No $C_{17:0}$	
Plant oil		
Virgin olive oil	No $C_{14:0}$, $C_{17:0} < 1\%$	

Table 24. Ratios of abundance of the straight-chain $C_{14:0}$ and $C_{17:0}$ fatty acid components in archaeological and ethnographic fats and oils.

Sample	$C_{14:0}/C_{17:0}$ fatty acid ratio
West Cotton	
RP91	4.0
WC30	5.2
RP22	4.3
RP2	2.7
RP4	1.0
RP28	1.8
RP73	2.3
RP30	3.6
RP60	2.4
RP78	1.4
RP6	1.3
RP10	1.8
RP88	1.3
RP72	2.1
RP89	1.0
RP83	1.0
Stanwick	
ST194	2.8
ST208	6.6
ST161	3.6
ST197	1.1
ST210	1.5
ST190	1.7
ST212	1.2
ST206bo	2.7
ST160	1.6
ST190bo	0.8
Wicken Bonhunt	
WKB16	1.3
WKB8	1.4
WKB15	1.6
WKB10	1.2
WKB1	0.8
WKB6	1.1
Botai	
i pot	0.8
N21 pot	1.5
N26 pot	1.0
Yarnton Cresswell Field	
104	No $C_{17:0}$ or $C_{14:0}$
128	2.8

Sample	$C_{14:0}/C_{17:0}$ fatty acid ratio
RP16	2.3
RP82	1.1
RP85	0.8
RP58	1.4
RP86	1.1
RP93	2.0
RP61	1.4
RP13	0.6
RP7	1.1
RP94	1.4
RP81	1.0
RP53	0.7
RP87	0.5
RP50	0.6
RP71	0.8
ST262	1.5
ST153	0.7
ST193	1.9
ST206r	0.9
ST152	0.8
ST211	0.3
ST156	0.8
ST215	0.5
ST226	0.7
WKB7	1.1
WKB2	1.1
WKB19	1.0
WKB13	0.6
WKB12	0.5
ii cr	1.2
xviii pot	1.9
iii cr	1.0
147	1.8
148	3.9

Table 24. Continued.

Sample	$C_{14:0}/C_{17:0}$ fatty acid ratio
121	4.7
114	No $C_{17:0}$ or $C_{14:0}$
129	0.2
136	No $C_{17:0}$ or $C_{14:0}$
112	0.4
137	4.0
127	2.5
146	3.3
141	4.7
130	4.9
144	0.9
Yarnton Flood Plain	
32	No $C_{17:0}$ or $C_{14:0}$
33	4.2
23	9.2
41	3.3
46b	No $C_{17:0}$ or $C_{14:0}$
38	No $C_{17:0}$ or $C_{14:0}$
5	3.23 % $C_{17:0}$, no $C_{14:0}$
Eton DBC	
28	2.2
22	2.2
21	1.6
20	2.0
9	0.4
13a	0.5
16	0.8
3	1.1
Eton LERW	
2163	2.3
865	2.1
2054	0.6
2166	0.9
2164	0.2
2271	0.2
2041-12	1.5
UpperNinepence	
P39 res	1.7
P38 res	1.0
P3	1.1
P68	0.2
P33 res	0.6
Ethnographic vessels	

Sample	$C_{14:0}/C_{17:0}$ fatty acid ratio
126	1.7
135	No $C_{17:0}$ or $C_{14:0}$
142	No $C_{17:0}$ or $C_{14:0}$
119	4.9
101	1.5
149	2.6
113	0.6
118	1.1
108	No $C_{17:0}$ or $C_{14:0}$
117	No $C_{17:0}$ or$_{14:0}$
21	1.4
44	8.5
40	No $C_{17:0}$ or $C_{14:0}$
31	0.6
49	1.4
1	3.07 % $C_{17:0}$, no $C_{14:0}$
50	2.1
25	0.4
1	0.6
19	0.8
11	0.3
7	0.3
8	0.3
13b	No $C_{17:0}$, 4.56 % $C_{14:0}$
12	0.1
2041-rim	0.6
571	No $C_{17:0}$ or $C_{14:0}$
601	0.3
2041-11	0.7
772	0.5
2041-8	1.0
1135	1.55 % $C_{17:0}$, no $C_{14:0}$
P66	0.1
P1b	1.0
P1a	0.1
P5	0.04

Table 24. Continued.

Sample	$C_{14:0}/C_{17:0}$ fatty acid ratio
Vessel A	3.3
Vessel B	3.9
Vessel C	No $C_{17:0}$ or $C_{14:0}$
Siberian horse fats	
Skin	4.8
Saccrum	10.8
Chest	1.2

Sample	$C_{14:0}/C_{17:0}$ fatty acid ratio
Vessel F	7.4
Vessel G	9.9

APPENDIX 4

APPENDIX 4. $C_{18:1}$ POSITIONAL AND GEOMETRIC ISOMER DISTRIBUTIONS

Table 1. $C_{18:1}$ isomers in modern fats (% abundance).

Sample	Δ^9		Δ^{10}		Δ^{11}		Δ^{12}		Δ^{13}		Δ^{14}		Δ^{15}	
	cis-	*trans-*	*cis-*	*trans-*	*cis-*	*trans-*	*cis-*	*trans-*	*cis-*	*trans-*	*cis-*	*trans-*	*cis-*	*trans-*
C1BB	85.6	3.0	0.3	0.4	2.0	5.5	0.2	0.5	0.3	0.6	0.1	0.8	0.2	0.4
C2BB	87.3	0.3	0.3	0.4	2.7	5.7	0.2	0.5	0.7	0.6	0.1	0.7	0.2	0.4
TULIP 1 MILK	88.7	nd	0.2	0.4	3.8	4.5	0.3	0.4	0.6	0.4	nd	0.5	0.1	0.3
TWINKLE 2 MILK	87.5	nd	0.4	0.9	3.8	5.1	0.2	0.5	0.4	0.4	nd	0.5	0.1	0.2
SARAH MILK 1	90.4	nd	nd	0.6	2.7	4.8	0.2	0.4	nd	0.4	nd	0.4	nd	0.2
RAM LAMB 2	87.8	nd	0.2	0.8	1.5	7.0	0.3	0.5	0.1	0.5	nd	0.7	0.1	0.4
MUTTON SHOULDER (EA. '98)	87.4	0.3	0.3	0.9	1.1	7.2	0.2	0.5	0.1	0.6	0.1	0.7	0.2	0.4
MUTTON LEG FAT (EA. '98)	86.7	nd	nd	0.7	0.6	8.2	0.2	0.5	0.1	0.7	0.1	1.0	0.3	0.8
HEB LAMB MILK 2	79.2	nd	nd	1.1	1.1	12.1	0.4	0.8	0.1	1.5	0.1	1.6	0.7	1.2
D1BR	87.7	0.3	0.4	0.9	2.4	6.3	0.2	0.3	0.5	0.3	0.1	0.3	0.1	0.2
D2BR	69.8	0.3	0.4	1.4	4.8	17.0	0.5	1.0	0.3	1.6	0.1	1.3	0.5	1.1
D3BR	70.8	nd	1.1	2.0	3.2	16.0	0.9	0.9	0.2	1.8	0.1	0.9	0.6	1.3
H4PL	93.4	0.6	0.3	0.8	4.2	0.2	nd	0.1	0.2	nd	nd	nd	nd	nd
H1PL	92.8	nd	0.4	0.6	5.9	0.1	nd	nd	0.2	nd	nd	nd	nd	nd
H5PP	93.7	nd	nd	0.4	5.6	nd	nd	0.1	0.2	nd	nd	nd	nd	nd
P2T121	90.8	nd	0.3	nd	8.5	nd	nd	nd	0.4	nd	nd	nd	nd	nd
P3T121	90.6	nd	nd	0.3	8.6	nd	0.1	nd	0.4	nd	nd	nd	nd	nd
P6T121	88.7	2.3	0.2	0.3	7.6	0.3	0.1	0.1	0.3	nd	nd	nd	0.1	nd
CHICKEN 1	89.0	3.6	0.2	0.3	6.3	0.4	nd	nd	0.1	nd	nd	nd	nd	nd
CHICKEN 2	95.5	nd	nd	nd	4.5	nd	nd	nd	nd	nd	nd	nd	nd	nd
CHICKEN 5	94.0	0.4	nd	0.1	5.2	nd	nd	nd	nd	0.1	nd	nd	nd	nd
GOOSE 1	95.8	nd	0.3	0.1	3.5	nd	nd	nd	0.3	nd	nd	nd	nd	nd
GOOSE 2	95.9	nd	0.3	0.2	3.5	nd	nd	nd	0.2	nd	nd	nd	nd	nd
GOOSE 3	97.2	nd	0.1	nd	2.5	nd	nd	nd	0.1	nd	nd	nd	nd	nd

Table 2. $C_{18:1}$ isomers in mixtures of modern fats (% abundance).

Sample	Δ^9		Δ^{10}		Δ^{11}		Δ^{12}		Δ^{13}		Δ^{14}		Δ^{15}	
	cis-	*trans-*	*cis-*	*trans-*	*cis-*	*trans-*	*cis-*	*trans-*	*cis-*	*trans-*	*cis-*	*trans-*	*cis-*	*trans-*
L3B91 100%	75.8	1.1	0.2	1.1	1.2	16.0	0.3	0.7	0.2	1.0	0.1	1.1	0.4	0.9
L3B91+P1T121 (75:25)	77.1	2.6	0.6	1.2	3.8	10.9	0.3	0.4	0.3	0.9	0.3	0.9	0.1	0.5
L3B91+P1T121 (50:50)	84.3	1.7	0.4	0.9	4.9	6.6	0.2	0.2	0.2	0.2	nd	0.2	0.1	0.1
C1BB+P1T121 (50:50)	92.7	nd	0.3	0.3	4.6	1.4	0.1	0.1	0.1	0.1	nd	0.1	nd	0.1
C1BB+P1T121 (25:75)	96.3	nd	0.4	nd	3.3	nd	nd	nd	nd	nd	nd	nd	nd	nd
L3B91+P1T121 (25:75)	91.0	nd	0.9	nd	5.9	2.2	nd	nd	nd	nd	nd	nd	nd	nd
L3B91+C1BB (50:50)	83.0	5.3	0.8	0.9	1.1	7.4	0.3	0.2	0.1	0.2	nd	0.3	0.1	0.2
C1BB+P1T121 (75:25)	89.5	1.6	0.5	0.6	3.6	2.9	0.2	0.2	0.2	0.2	nd	0.2	0.1	0.2

Table 3. $C_{18:1}$ isomers in Siberian horse fats (% abundance).

Sample	Δ^9		Δ^{10}		Δ^{11}		Δ^{12}		Δ^{13}		Δ^{14}		Δ^{15}	
	cis-	*trans-*	*cis-*	*trans-*	*cis-*	*trans-*	*cis-*	*trans-*	*cis-*	*trans-*	*cis-*	*trans-*	*cis-*	*trans-*
SKIN (H1)	38.1	12.9	3.5	10.0	6.1	22.3	nd	0.4	0.2	0.2	nd	0.5	4.9	0.8
SACCRUM (H1)	41.3	9.7	4.1	8.5	9.5	21.4	0.5	0.9	0.4	0.8	0.1	0.9	1.1	0.7

Table 4. $C_{18:1}$ isomers in lipid extracts from ethnographic vessels (% abundance).

Sample	Δ^9		Δ^{10}		Δ^{11}		Δ^{12}		Δ^{13}		Δ^{14}		Δ^{15}	
	cis-	*trans-*	*cis-*	*trans-*	*cis-*	*trans-*	*cis-*	*trans-*	*cis-*	*trans-*	*cis-*	*trans-*	*cis-*	*trans-*
RFETP1 (POT A)	89.1	1.2	0.5	0.9	6.9	0.4	0.1	0.2	0.3	0.1	nd	0.1	nd	0.1
RFETB1 (POT B)	43.5	4.1	0.2	4.1	4.9	30.6	0.5	3.0	nd	3.6	nd	3.5	0.3	1.9
RFETO1 (POT C)	96.7	0.7	nd	0.1	2.5	nd	nd	nd	nd	nd	nd	nd	nd	nd
RFETM1 (POT F)	85.9	5.5	0.2	0.4	7.1	0.6	nd	nd	0.3	0.1	nd	nd	nd	nd

Table 5. $C_{18:1}$ isomers in lipid extracts from archaeological vessels from West Cotton, Northamptonshire (% abundance).

Sample	Δ^9		Δ^{10}		Δ^{11}		Δ^{12}		Δ^{13}		Δ^{14}		Δ^{15}	
	cis-	*trans-*	*cis-*	*trans-*	*cis-*	*trans-*	*cis-*	*trans-*	*cis-*	*trans-*	*cis-*	*trans-*	*cis-*	*trans-*
RP 2	45.3	24.5	1.0	5.9	6.9	10.2	0.5	1.3	0.5	1.3	0.3	1.2	0.3	0.9
RP 4	56.4	25.7	0.4	3.9	6.5	4.6	0.2	0.4	0.4	1.0	0.1	0.3	0.1	0.2
RP 6	14.2	19.8	2.6	20.9	3.1	21.2	1.2	7.3	1.3	4.4	0.2	2.4	0.2	1.3
RP 7	23.3	34.2	0.5	4.1	3.4	29.9	0.3	1.4	0.1	1.2	nd	0.9	nd	0.6
RP 16	23.3	5.5	0.2	4.7	nd	47.8	0.4	3.3	0.1	4.7	0.1	5.3	0.6	4.1
RP 50	56.4	5.3	0.3	2.2	4.4	21.0	0.4	1.4	0.4	2.3	0.1	3.0	0.5	2.5
RP 58	42.9	9.8	0.5	2.4	2.5	28.4	0.6	2.5	0.2	3.3	0.2	3.7	0.5	2.5
RP 60	20.5	10.2	0.5	5.9	1.8	41.4	0.6	3.6	0.4	4.8	0.3	5.5	0.6	3.8
RP 71	14.2	5.0	0.2	4.8	nd	59.7	0.4	3.3	0.1	4.5	0.1	4.5	0.3	2.9
RP 89	0.3	14.3	0.1	18.2	0.5	19.5	nd	11.5	0.1	11.8	0.7	12.1	1.0	9.8
RP 91	39.1	5.2	nd	4.7	nd	31.0	0.9	3.0	0.3	4.8	0.1	5.6	1.1	4.2
RP 94	13.2	16.7	0.6	8.3	0.7	45.1	0.5	3.7	0.1	3.7	0.1	4.1	0.2	2.9
RP 53	48.8	12.6	0.4	2.6	2.1	27.1	0.5	1.4	0.3	1.4	0.1	1.5	0.2	1.2
RP 86	7.5	22.2	0.5	3.1	0.9	47.9	0.3	2.8	0.2	4.1	0.1	5.5	0.6	4.3
RP 93	8.3	7.8	0.2	8.7	nd	48.5	0.3	6.2	0.2	6.5	0.2	7.2	0.6	5.4
RP 78	28.3	21.6	0.3	4.1	1.4	30.2	0.3	2.8	0.3	3.2	nd	4.0	0.3	3.2
RP 82	12.4	22.2	0.5	23.0	1.6	26.5	0.5	4.9	0.2	3.3	0.1	3.0	0.2	1.8
RP 83	51.7	6.0	0.5	4.7	3.2	21.1	0.5	2.4	0.3	2.9	0.2	3.1	0.6	2.6
RP 87	30.1	11.0	0.3	2.9	2.5	32.7	0.4	3.1	0.4	4.7	0.2	5.7	0.9	5.2
RP 30	29.3	6.4	0.5	3.2	1.5	42.7	0.4	3.0	0.3	3.8	0.2	4.5	0.9	3.3
RP 72	1.5	28.2	0.7	8.3	1.9	36.2	0.3	5.3	0.3	5.7	0.3	6.4	0.4	4.4
WC30	26.1	17.4	3.2	2.5	19.2	16.6	2.1	1.7	1.8	1.7	2.4	2.1	1.7	1.5

Table 6. $C_{18:1}$ isomers in lipid extracts from archaeological vessels from Stanwick, Northamptonshire (% abundance).

Sample	Δ^9		Δ^{10}		Δ^{11}		Δ^{12}		Δ^{13}		Δ^{14}		Δ^{15}	
	cis-	*trans-*	*cis-*	*trans-*	*cis-*	*trans-*	*cis-*	*trans-*	*cis-*	*trans-*	*cis-*	*trans-*	*cis-*	*trans-*
ST190	33.9	n[1]	1.1	14.9	3.6	28.3	0.8	5.8	0.9	5.4	0.6	4.6	nd	0.1
ST194	13.2	10.4	0.6	4.8	6.0	48.3	0.7	3.3	0.7	4.4	0.4	4.3	0.5	2.3
ST 206 RIM	29.3	15.6	1.7	11.0	2.3	24.1	0.7	4.2	0.6	3.3	0.3	3.7	0.6	2.5
ST206 BODY	18.5	17.2	1.4	8.0	4.1	34.3	0.8	3.7	0.6	3.6	0.4	4.1	0.5	2.7
ST210	29.4	13.9	0.5	5.4	2.9	33.4	0.4	3.1	0.5	4.1	0.3	3.7	0.5	2.0
ST211	15.9	31.1	1.5	3.1	11.7	24.5	1.0	1.9	1.2	2.3	0.9	2.5	0.8	1.8
ST212	52.8	11.3	0.4	2.0	3.5	22.9	0.4	1.4	0.3	1.6	0.1	1.9	0.2	1.3

[2] n=not measured

Table 7. $C_{18:1}$ isomers in lipid extracts from archaeological vessels from Wicken Bonhunt, Essex (% abundance).

Sample	Δ^9		Δ^{10}		Δ^{11}		Δ^{12}		Δ^{13}		Δ^{14}		Δ^{15}	
	cis-	*trans-*	*cis-*	*trans-*	*cis-*	*trans-*	*cis-*	*trans-*	*cis-*	*trans-*	*cis-*	*trans-*	*cis-*	*trans-*
WKB 2	73.1	2.8	nd	1.5	3.6	14.7	0.4	1.1	nd	0.9	nd	1.0	nd	1.1
WKB 3	91.6	1.0	nd	1.2	2.7	2.4	0.2	0.2	0.1	0.2	nd	0.2	0.1	0.2
WKB 7	67.9	7.3	0.7	3.8	5.1	10.1	0.4	0.9	0.4	0.9	0.1	1.1	0.3	0.8
WKB 8	73.8	11.7	0.4	2.7	4.1	4.4	0.1	0.3	0.3	0.6	0.1	0.6	0.2	0.6
WKB 10	84.4	nd	nd	2.1	nd	8.1	nd	1.2	nd	1.9	nd	0.7	nd	1.6
WKB 12	72.0	2.8	5.4	4.0	8.4	1.0	1.4	0.4	1.4	0.2	1.1	nd	1.9	nd
WKB 15	63.0	4.8	0.6	5.0	8.3	7.1	0.8	2.2	1.9	2.1	0.5	1.7	0.8	1.2
WKB 16	83.2	1.6	0.3	1.5	9.2	2.2	0.1	0.3	0.6	0.3	nd	0.3	0.1	0.2

Table 8. $C_{18:1}$ isomers in laboratory degraded lamb fats (% abundance).

Sample	Δ^9		Δ^{10}		Δ^{11}		Δ^{12}		Δ^{13}		Δ^{14}		Δ^{15}	
	cis-	*trans-*	*cis-*	*trans-*	*cis-*	*trans-*	*cis-*	*trans-*	*cis-*	*trans-*	*cis-*	*trans-*	*cis-*	*trans-*
AER AUT 1	73.7	nd	0.8	2.0	1.7	18.1	0.5	0.8	0.3	0.9	nd	0.8	nd	0.5
EV6 B BASE 1	87.1	nd	nd	0.8	nd	11.5	nd	0.1	nd	0.1	nd	0.2	nd	0.1
EV6 B BASE 2	87.9	nd	nd	0.9	nd	10.1	nd	0.3	0.1	0.2	nd	0.3	nd	0.2
AN 1	85.4	nd	nd	0.6	nd	13.4	nd	0.2	nd	0.2	nd	0.2	nd	nd
EV6 SB3 1	80.2	6.5	nd	1.2	nd	7.9	0.6	0.4	nd	0.5	nd	0.4	nd	nd

Table 9. $C_{18:1}$ isomers in laboratory degraded olive oil (% abundance).

Sample	Δ^9		Δ^{10}		Δ^{11}		Δ^{12}		Δ^{13}		Δ^{14}		Δ^{15}	
	cis-	*trans-*	*cis-*	*trans-*	*cis-*	*trans-*	*cis-*	*trans-*	*cis-*	*trans-*	*cis-*	*trans-*	*cis-*	*trans-*
Olive oil D0	97.9	nd	nd	nd	2.1	nd	nd	nd	nd	nd	nd	nd	nd	nd
Olive oil D90 T2! degn	97.9	nd	nd	nd	2.1	nd	nd	nd	nd	nd	nd	nd	nd	nd

APPENDIX 5

APPENDIX 5. TRIACYLGLYCEROL DISTRIBUTIONS IN MODERN AND ARCHAEOLOGICAL FATS AND R_f VALUES FOR SILVER ION TLC OF MODERN FATS AND OLIVE OIL.

Table 1. R_f values for fractions in reference fats and olive oil separated by silver ion TLC.

Reference fat/oil							
Olive oil		**Goose adipose**		**Cow adipose**		**Ewe adipose**	
Spot no.	**R_f value**	**Spot no.**	**R_f value**	**Spot no.**	**R_f value**	**Spot no.**	**R_f value**
26	0.74	14	0.74	8	0.74	15	0.72
25	0.58	13	0.52	7	0.65	14	0.62
24	0.36	12	0.45	6	0.56	13	0.54
23	0.19	11	0.22	5	0.45	12	0.48
22	0.05	10	0.09	4	0.38	11	0.44
		9	0.03	3	0.23	10	0.40
				2	0.13	9	0.34
				1	0.05	8	0.26
						6	0.15
						5	0.07

Table 1. Continued.

Reference fat							
Chicken adipose		**Pig adipose**		**Deer adipose**		**Horse adipose**	
Spot no.	**R_f value**	**Spot no.**	**R_f value**	**Spot no.**	**R_f value**	**Spot no.**	**R_f value**
21	0.71	26	0.72	8	0.76	21	0.74
20	0.54	25	0.50	7	0.67	20	0.52
19	0.46	24	0.29	6	0.46	19	0.45
18	0.27	22	0.11	5	0.39	18	0.22
16	0.11			4	0.24	17	0.13
				3	0.14	16	0.09
				2	0.09	15	0.06
				1	0.06		

Table 2. Triacylglycerol distributions (% abundance) in reference fats and olive oil fractions separated by silver ion TLC.

Fat/oil	TLC fraction no.	Triacylglycerol (C_n)[1]							
		C_{42}	C_{44}	C_{46}	C_{48}	C_{50}	C_{52}	C_{54}	C_{56}
OLIVE OIL	25	nd	nd	nd	0.4	50.5	37.0	10.6	1.4
GOOSE FAT	14	nd	nd	3.3	54.6	34.2	7.7	0.3	nd
	13	nd	nd	nd	4.2	68.6	24.9	2.3	nd
C2BB	8	nd	3.1	16.3	32.4	30.2	14.7	3.3	nd
	7	nd	nd	2.7	22.2	37.8	29.0	8.3	nd
EWE	15	nd	nd	4.1	15.2	29.5	33.6	17.7	nd
	14	nd	nd	nd	5.5	21.9	41.9	30.7	nd
CHICKEN	21	nd	nd	3.7	47.7	38.6	10.0	nd	nd
	20	nd	nd	nd	3.8	61.6	31.0	3.6	nd
P1T121	26	nd	0.6	2.2	13.5	38.7	42.1	2.9	nd
	25	nd	nd	0.4	4.4	28.9	62.0	4.3	nd
	24	nd	nd	nd	1.0	13.2	75.0	10.5	0.3
D3BR	8	nd	0.4	3.4	14.4	31.6	35.4	14.8	nd
	7	nd	nd	0.3	4.0	23.6	46.9	25.2	nd
H1PL	20	nd	nd	1.6	18.6	45.3	30.8	3.7	nd
	21	nd	0.9	7.1	30.3	38.7	18.7	4.3	nd

[1] C_n=no. of acyl carbon atoms.

Table 3. Distributions of triacylglycerols (% abundance) in lipid extracts from vessels from West Cotton, Northamptonshire.

	Triacylglycerol (C_n)[1]							
Sample	C_{40}	C_{42}	C_{44}	C_{46}	C_{48}	C_{50}	C_{52}	C_{54}
RP2	nd	nd	1.3	3.4	16.6	27.4	40.8	10.4
RP4RIMRA	nd	nd	0.3	1.4	9.2	29.1	44.8	15.2
RP6RA	nd	nd	nd	2.2	17.9	34.2	33.9	11.7
RP7BODR	nd	nd	nd	0.9	5.8	19.9	43.6	29.8
RP10R	nd	nd	1.3	0.6	27.1	18.0	34.8	18.2
RP13BODR	nd	nd	1.1	3.0	16.3	29.6	33.9	16.2
RP16BOI	nd	nd	nd	1.6	7.1	23.7	39.9	27.7
RP22BASR	1.0	4.7	7.4	12.0	19.9	28.5	20.8	5.7
RP28BBR	nd	nd	1.6	2.8	10.1	27.2	42.5	15.8
RP30BBAR	nd	1.0	2.9	5.7	11.5	26.9	34.9	17.1
RP50BO2	nd	nd	0.8	3.3	12.4	29.3	38.6	15.5
RP53BUB	nd	nd	0.2	1.6	8.4	25.7	41.3	22.8
RP60ABAS	0.4	2.6	4.7	8.3	15.5	26.1	29.0	13.4
RP61RIM	2.1	5.8	8.9	11.6	15.1	23.4	22.7	10.4
RP71BAB	nd	nd	0.4	1.4	7.9	25.3	41.0	24.0
RP72RIMA	0.8	1.6	3.0	6.1	15.4	28.0	30.7	14.4
RP73ARIM	nd	2.2	4.4	7.6	18.8	31.5	27.8	7.8

Table 3. Continued.

	Triacylglycerol (C_n)[1]							
Sample	C_{40}	C_{42}	C_{44}	C_{46}	C_{48}	C_{50}	C_{52}	C_{54}
RP78RIMB	nd	nd	1.0	4.2	15.6	34.0	33.0	12.2
RP81BOBR	nd	nd	0.7	1.7	7.9	24.5	40.5	24.6
RP82RIM	1.2	2.5	5.3	7.0	14.8	26.2	30.7	12.3
RP83RI95	nd	nd	nd	0.7	7.8	23.8	45.5	22.2
RP85RIM	nd	nd	0.7	1.7	9.1	26.1	39.5	23.0
RP86BODY	1.9	5.1	6.9	10.3	17.7	27.0	18.2	13.0
RP87RI95	nd	nd	nd	1.0	8.2	27.8	43.5	19.5
RP88BO95	nd	nd	nd	nd	5.5	29.0	57.6	7.8
RP89ALB	nd	nd	1.0	5.5	19.1	32.7	30.2	11.5
RP91RI95	nd	3.3	6.2	13.7	23.2	27.6	21.6	4.4
94RBCO95	nd	1.0	2.6	6.0	13.5	27.1	34.1	15.8
WC30	0.9	2.8	5.2	9.3	16.4	27.2	26.6	11.7

[1] C_n=no. of acyl carbon atoms.

Table 4. Distributions of triacylglycerols (% abundance) in lipid extracts from vessels from Stanwick, Northamptonshire.

	Triacylglycerol (C_n)[1]								
Sample	C_{38}	C_{40}	C_{42}	C_{44}	C_{46}	C_{48}	C_{50}	C_{52}	C_{54}
215	nd	nd	nd	nd	0.7	8.3	24.0	39.0	28.0
153bri	nd	nd	nd	0.7	4.0	11.3	24.6	36.9	22.5
262	nd	nd	nd	1.4	5.1	18.5	30.1	34.4	10.6
152bli	nd	nd	nd	nd	1.6	8.3	26.5	40.4	23.2
156bri	nd	nd	nd	1.1	2.8	11.6	27.7	36.8	19.9
211rim	nd	nd	nd	0.2	1.1	7.2	24.1	40.1	27.3
212	nd	nd	nd	0.3	5.3	20.4	34.2	30.0	9.7
161bod	nd	2.6	6.7	9.8	14.3	17.7	22.2	19.4	7.2
160base	nd	1.7	5.1	8.2	15.1	22.9	25.7	16.8	4.5
226rb	nd	nd	nd	nd	4.5	19.5	38.0	29.9	8.1
197	nd	1.4	3.3	4.5	7.4	14.2	27.1	28.8	13.3
194	1.5	3.8	8.5	9.8	13.9	18.2	21.0	18.4	5.0
193b	nd	nd	nd	nd	2.3	14.6	30.6	39.6	13.0
190rim	nd	nd	nd	nd	1.5	9.4	28.6	41.8	18.7
190bod	nd	nd	nd	0.9	2.7	9.9	27.2	43.1	16.2
210bod	nd	nd	0.8	2.0	6.1	16.8	28.8	31.7	13.8
208	nd	2.2	5.5	10.4	16.2	21.7	21.5	17.1	5.3
206rim	nd	nd	nd	1.1	2.7	10.2	27.2	37.5	21.3
206bod	nd	0.7	4.8	5.5	8.9	15.4	26.0	26.0	12.5

Table 5. Distributions of triacylglycerols (% abundance) in lipid extracts from vessels from Wicken Bonhunt, Essex.

	Triacylglycerol (C_n)[1]							
Sample	C_{40}	C_{42}	C_{44}	C_{46}	C_{48}	C_{50}	C_{52}	C_{54}
1	0.0	0.0	1.1	2.1	11.8	33.7	37.3	14.1
2	0.0	0.0	0.0	2.1	11.2	28.3	39.6	18.8
3	0.0	0.0	1.3	0.7	7.1	28.6	43.6	18.6
6	0.0	0.0	0.0	2.4	14.9	34.6	35.0	13.1
7	0.0	0.0	0.0	1.7	9.0	29.5	39.9	19.9
8	0.0	0.0	0.0	1.3	6.6	24.8	52.5	14.7
10	0.0	0.0	0.0	4.1	11.1	27.6	36.1	21.1
12	0.0	0.0	1.3	1.2	10.2	30.6	37.5	19.2
13	0.0	0.0	1.0	1.6	9.3	29.8	45.7	12.5
14	0.0	0.0	0.0	1.7	10.0	29.8	38.6	20.0
15	0.0	0.0	0.0	2.2	11.9	31.9	38.3	15.7
16	0.0	0.0	0.0	2.2	9.7	31.0	46.7	10.4
18	0.0	0.0	0.0	1.1	7.6	24.6	44.8	21.9
19R	0.0	0.0	0.0	1.3	7.8	24.9	39.8	26.3

Table 6. Distributions of triacylglycerols (% abundance) in lipid extracts from vessels from Botai, Kazakhstan.

	Triacylglycerol (C_n)[1]							
Sample	C_{40}	C_{42}	C_{44}	C_{46}	C_{48}	C_{50}	C_{52}	C_{54}
XVIII (pot)	nd	nd	nd	5.2	18.2	28.0	27.4	21.1
N26 (pot)	nd	nd	nd	5.1	22.9	36.2	26.4	9.4

Table 7. Distributions of triacylglycerols (% abundance) in lipid extracts from vessels from Yarnton Cresswell field, Oxfordshire.

	Triacylglycerol (C_n)[1]								
Sample	C_{40}	C_{42}	C_{44}	C_{46}	C_{48}	C_{50}	C_{52}	C_{54}	C_{56}
104	0.0	0.0	5.2	11.7	23.8	27.1	22.1	10.2	0.0
109	0.0	0.0	0.0	1.7	8.2	24.3	37.9	27.8	0.0
112	0.0	0.0	0.0	1.0	6.7	24.0	44.2	24.1	0.0
113	0.0	0.0	0.0	0.0	8.0	29.7	43.1	19.2	0.0
114	0.0	0.0	0.0	8.5	15.8	28.8	31.9	15.0	0.0
118	0.0	0.0	9.2	9.6	16.9	29.8	24.6	9.9	0.0
119	0.0	0.0	0.0	4.0	11.4	28.4	37.5	18.6	0.0
121	0.0	2.4	7.5	15.1	25.7	27.5	17.2	4.8	0.0
126	0.9	2.7	3.3	4.7	13.1	25.7	33.2	15.2	1.1
127	0.9	2.7	6.5	11.6	19.5	27.0	22.1	8.0	1.6
128	0.0	2.7	6.2	12.3	20.8	28.1	22.4	7.4	0.0

Table 7. Continued.

	Triacylglycerol (C_n)[1]								
Sample	C_{40}	C_{42}	C_{44}	C_{46}	C_{48}	C_{50}	C_{52}	C_{54}	C_{56}
129	1.4	3.2	6.6	13.0	22.5	26.0	19.7	6.8	0.9
130	0.0	0.0	11.5	14.6	23.0	24.6	18.7	7.6	0.0
136	0.0	0.0	7.6	6.4	14.0	27.1	28.5	16.4	0.0
137	0.7	2.5	4.9	10.3	17.1	25.8	25.5	11.5	1.7
141	0.0	0.6	3.1	6.7	14.9	28.3	30.2	14.5	1.8
144	0.0	0.0	0.0	0.0	0.0	15.1	42.0	42.9	0.0
146	0.3	2.9	7.0	11.3	19.3	28.4	23.8	6.9	0.0
148	0.0	0.0	0.0	1.0	10.1	29.7	37.5	21.8	0.0

Table 8. Distributions of triacylglycerols (% abundance) in lipid extracts from vessels from Yarnton flood plain, Oxfordshire.

	Triacylglycerol (C_n)[1]								
Sample	C_{40}	C_{42}	C_{44}	C_{46}	C_{48}	C_{50}	C_{52}	C_{54}	C_{56}
1	nd	0.9	1.9	4.6	14.3	30.0	34.7	13.8	nd
2	nd	nd	nd	nd	29.6	26.7	30.9	12.8	nd
4	1.3	3.8	7.3	13.6	21.6	26.3	18.7	5.8	1.5
5	0.7	1.6	4.3	9.1	16.3	25.6	28.2	10.8	3.4
11	nd	nd	nd	nd	58.6	18.8	22.6	nd	nd
12	nd	nd	nd	nd	62.2	24.8	6.9	6.1	nd
13	nd	nd	nd	nd	72.0	28.0	nd	nd	nd
14	nd	nd	nd	nd	63.5	15.5	12.5	8.6	nd
15	nd	nd	nd	nd	68.7	21.3	6.3	2.4	1.3
16	nd	nd	nd	nd	43.3	16.5	16.9	23.4	nd
17	nd	nd	nd	nd	69.5	24.3	6.2	nd	nd
18	nd	nd	nd	nd	77.1	22.9	nd	nd	nd
21	nd	nd	nd	8.7	39.7	28.5	16.2	6.8	nd
23	1.6	2.7	6.8	12.3	20.8	22.1	25.3	8.4	nd
24	nd	nd	5.4	6.9	20.6	27.6	27.8	11.8	nd
30	nd	nd	7.7	10.7	16.6	24.7	25.5	14.7	nd
31	0.3	0.9	3.1	8.2	18.5	28.5	25.0	7.8	7.7
38	nd	nd	nd	nd	8.1	34.1	53.0	4.7	nd
40	nd	nd	nd	nd	27.7	36.4	24.9	11.0	nd
41	nd	3.7	7.2	8.2	14.7	22.8	26.1	17.2	nd
43	nd	nd	nd	nd	10.1	30.4	42.7	16.8	nd
44	nd	nd	nd	7.6	16.9	33.6	34.4	7.6	nd
49	nd	nd	3.2	4.1	11.5	26.1	34.9	20.1	nd
50	nd	nd	nd	8.6	19.3	29.1	32.8	10.1	nd

Table 9. Distributions of triacylglycerols (% abundance) in lipid extracts from vessels from Eton Lake End Road, Oxfordshire.

		Triacylglycerol (C_n)[1]								
Sample no.	**context/ small find No.**	C_{40}	C_{42}	C_{44}	C_{46}	C_{48}	C_{50}	C_{52}	C_{54}	C_{56}
NRA2	959/2014-8	nd	2.1	7.6	9.3	18.4	28.2	25.7	7.9	0.9
NRA2	959/2041-11	1.5	4.5	11.4	13.6	19.7	26.3	22.3	0.8	nd
NRA2	959/2041-12	nd	1.3	4.4	9.6	21.5	30.7	24.7	6.9	0.9
NRA2	959/2041-rim	nd	1.5	5.7	9.6	19.0	29.1	26.6	8.7	nd
NRA3	1224/2272	nd	nd	11.9	9.3	20.4	29.0	22.4	7.1	nd
NRA4	1224/2271	nd	nd	nd	1.0	6.6	27.1	53.8	11.5	nd
NRA5	1224/2208	nd	nd	13.5	9.0	18.3	26.5	26.3	6.4	nd
NRA8	606/2163	nd	nd	nd	8.5	18.8	32.6	37.3	2.7	nd
NRA8	606/2164	nd	nd	nd	nd	10.1	26.6	49.9	13.4	nd
NRA8	606/2166	nd	nd	nd	3.8	12.9	34.7	39.3	9.4	nd
NRA10	529/865	nd	1.1	27.8	6.9	16.1	24.2	18.1	5.9	nd
NRA11	685/601	nd	nd	6.2	7.6	14.7	25.1	24.5	21.9	nd

Table 10. Distributions of triacylglycerols (% abundance) in lipid extracts from vessels from Eton Rowing Lake, Oxfordshire.

	Triacylglycerol (C_n)[1]							
Sample	C_{40}	C_{42}	C_{44}	C_{46}	C_{48}	C_{50}	C_{52}	C_{54}
DBC1	nd	nd	0.4	1.3	8.4	24.9	39.5	25.4
DBC2	nd	nd	11.1	13.0	24.1	27.8	20.1	4.0
DBC3	nd	2.2	8.9	9.6	21.1	28.3	22.6	7.3
DBC8	nd	nd	2.4	5.5	18.0	29.4	33.1	11.6
DBC9	nd	nd	nd	12.0	23.5	33.7	19.4	11.4
DBC11	nd	1.5	3.3	9.0	21.5	31.5	24.9	8.2
DBC12	nd	nd	nd	nd	14.2	31.4	37.6	16.8
DBC13A	nd	nd	nd	5.2	14.4	32.5	34.4	13.6
DBC13B	nd	nd	5.1	14.1	11.8	23.7	25.0	20.3
DBC16	nd	1.2	3.2	11.7	19.6	28.1	25.2	11.1
DBC19	nd	nd	3.0	10.6	23.2	32.5	27.7	3.0
DBC20	nd	1.7	4.1	8.2	17.3	30.9	29.7	8.2
DBC21	0.2	1.7	4.6	11.1	23.2	30.8	21.5	6.9
DBC22	nd	nd	2.4	7.6	40.0	29.5	17.6	3.0
DBC25	nd	3.7	5.5	9.8	19.3	29.2	23.8	8.8
DBC28	2.0	6.0	12.7	14.4	21.6	24.0	16.5	2.7

Table 11. Distributions of triacylglycerols (% abundance) in lipid extracts from vessels from Walton, mid Wales.

	Triacylglycerol (C_n)[1]								
Sample	C_{40}	C_{42}	C_{44}	C_{46}	C_{48}	C_{50}	C_{52}	C_{54}	C_{56}
P39 (cr)	3.5	5.9	9.9	13.9	22.1	25.2	12.4	5.5	1.7
P38 (pot)	nd	4.2	6.3	10.3	27.0	26.7	16.1	9.4	nd
P38 (cr)	1.7	3.0	7.9	13.1	21.8	27.0	19.3	6.1	nd

Table 12. Distributions of triacylglycerols (% abundance) in degraded fats of known animal origin.

	Triacylglycerol (C_n)[1]							
Sample	C_{40}	C_{42}	C_{44}	C_{46}	C_{48}	C_{50}	C_{52}	C_{54}
Pork (ethnographic vessel A)	nd	nd	nd	0.5	3.4	23.1	55.7	17.2
Lamb D40(degradation expt.; Charters, 1996)	nd	0.9	2.0	3.9	9.0	19.7	38.5	26.0

Table 13. Distributions of triacylglycerols (% abundance) in Siberian horse fats/tissues.

	Triacylglycerol (C_n)[1]							
Sample	C_{40}	C_{42}	C_{44}	C_{46}	C_{48}	C_{50}	C_{52}	C_{54}
H1STOMLI	nd	nd	nd	10.8	30.6	33.6	21.3	3.8
H1SKIN	nd	nd	0.9	11.3	31.1	33.8	19.9	2.8
h2sacvert	nd	nd	3.3	10.3	19.5	26.5	27.9	12.5

APPENDIX 6

APPENDIX 6. $\delta^{13}C$ VALUES OF FATTY ACIDS IN MODERN AND ARCHAEOLOGICAL FATS AND OILS AND BULK DATA FOR ANIMAL FEEDS.

Table 1. $\delta^{13}C$ values[1] (‰) for individual fatty acids in modern ruminant fats.

	Fatty acid							
Sample	$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
Cows adipose								
C1BB		-29.3	-29.3	-32.2	-32.0			
C1BR		-29.7	-30.2	-32.7	-32.0			
C1BK		-29.1	-29.1	-31.9	-31.6			
C2BB		-32.6	-30.3	-32.0	-30.9	-36.7	-33.2	
Cows milk								
Mallard millk (first sample)		-27.2	-28.2	-32.4				
Mallard milk (post-birth)		-27.2	-31.2	-34.8	-33.1			
Sarah milk (pre-birth)		-28.8	-28.5	-34.4	-34.2			
Sarah milk (post-birth)		-27.9	-27.8	-32.6	-30.5			
Tulip 2 milk		-28.5	-29.0	-34.5	-33.1	-36.7	-34.5	
Twinkle 2 milk (25th Feb 1998)		-28.4	-30.0	-35.3	-33.1	-36.5	-34.6	-32.5
Twinkle 2 (early Feb 1998)		-27.4	-28.3	-33.5	-32.0	-35.2		
Tulip 1 milk		-28.8	-29.3	-34.1	-31.9	-39.4	-35.1	
Sheep adipose								
L1B91		-28.6	-29.8	-31.6	-30.7		-37.5	
L1K91		-28.0	-29.4	-30.8	-30.7		-41.5	
L2B91		-28.7	-29.8	-32.2	-31.3		-40.7	-33.4
L2K91		-28.6	-30.9	-32.9	-31.9		-36.1	
L3B91		-30.3	-30.6	-32.7	-30.6		-56.3	-35.0
L3K91		-30.3	-30.8	-32.6	-35.8		-50.6	
E8B91 (ewe orig.)			-28.6	-30.4				
Mutton fat		-39.4	-29.2	-30.5	-28.6			
Hebridean lamb		-27.5	-28.8	-31.7	-30.6			
Mutton leg fat		-28.9	-29.7	-31.5	-30.2	-38.8	-35.4	-31.5
Mutton shoulder fat		-28.9	-29.6	-31.5	-30.0	-36.5	-31.4	
Ram lamb 1 (10 months)		-30.8	-29.1	-30.8				
Ram lamb 2 (10 months)		-28.1	-28.8	-30.5	-29.2	-38.8	-33.2	-32.9
Sheep milk								

Table 1 Continued

Heb lamb (post-birth)		-26.2	-29.4	-33.8	-31.6			
Hebridean lamb 2	-33.4	-26.2	-29.8	-34.2	-33.0		-35.6	
Deer fat								
D1BK		-28.7	-30.1					
D1BB		-29.2	-30.9					
D1BR		-29.8	-30.1					
D2BR		-29.2	-32.5					
D2BB		-28.4	-33.6					
D3BR		-31.1	-34.2					
D3BK		-31.2	-34.0					

[1] Data has been corrected for the change in $\delta^{13}C$ of atmospheric CO_2 which has occurred since the Industrial Revolution.

Table 2. $\delta^{13}C$ values[1] (‰) for individual fatty acids in modern non-ruminant fats and oils.

	Fatty acid						
Sample	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
Goose fat							
Goose 1		-29.1	-27.7	-28.2		-32.9	
Goose 2		-29.1	-28.4	-28.1		-33.3	
Goose 3		-29.1	-28.2	-28.2		-33.1	
Goose 4		-30.1	-28.5	-28.7		-33.0	
Chicken fat							
Chicken 1		-29.3	-28.6	-26.9			
Chicken 2		-29.1	-28.0	-27.6	-31.2	-25.7	
Chicken 3		-29.0	-28.3	-27.5	-31.8	-28.5	
Chicken 4		-28.9	-28.3	-27.7	-31.2	-29.3	-29.2
Chicken 5	-32.9	-29.0	-28.6	-27.6	-32.7	-27.6	
Chicken 6		-28.9	-28.2	-27.7	-31.6	-28.7	
Chicken 8		-29.4	-27.9	-27.9	-32.2	-29.1	-29.3
Chicken 9		-28.4	-27.7	-24.9			
Pig fat							
P1T121 (rep.)		-25.2	-24.4				
P2T121	-26.8	-26.8	-25.2	-25.2	-28.6	-30.3	
P3T121	-27.9	-26.9	-25.2	-25.5	-28.1	-30.3	
P4T121	-27.0	-25.9	-24.8	-24.8	-29.8		
P5T121	-27.3	-26.4	-25.3	-25.1	-29.5	-30.4	
P6T121	-27.5	-25.5	-24.2	-24.9	-29.6	-31.9	
P7T121	-27.6	-26.1	-24.8	-25.9	-29.8		
P8T121	-27.3	-25.8	-24.5	-25.1	-29.3	-29.9	
Andy Stott C_3 pig		-24.8	-25.7	-25.6		-30.1	
Horse fat							
H1PL	-30.8	-30.1	-29.8	-29.7			
H3PP		-31.0	-30.5				
H3PL		-30.9	-30.0				
H4PP		-30.4	-29.6				
H4PL		-30.7	-30.3				
H5PP		-29.9	-30.0				
H5PL		-30.0	-27.9				
H6PL	-31.8	-30.3	-30.1	-29.5			
Fish							
Cod fillet fame		-24.9	-26.2	-27.6			
Haddock fillet		-26.2	-23.9	-24.0			
Plaice fillet		-24.1	-23.5	-23.5			
Virgin olive oil		-28.5	-28.5	-26.4			

Table 3. $\delta^{13}C$ values[1] (‰) for individual fatty acids in the diets of reference animals.

	Fatty acid					
Sample	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
Cows grass (Brockley)	-38.0	-34.1	-31.5	-25.3	-30.6	
Deer grass (Brockley)	-33.5	-31.5	-28.7	-24.5	-29.5	
Sheep grass (Baker)	-34.7	-35.2	-33.3	-25.5	-31.2	
Pig feed (Baker)		-30.9	-30.3	-28.3		
Silage (Baker)	-34.7	-33.3	-32.4	-27.3	-29.8	
Ruminant feed (Baker)	-24.2	-27.6	-26.7	-24.4	-27.6	
Goose grass		-32.9	-30.7	-29.1	-32.5	
Goose pellets		-34.2	-31.6	-30.8	-32.3	
Chicken feed	-28.4	-30.6	-29.3	-28.1	-29.1	-30.4

Table 4. Bulk $\delta^{13}C$ values[1] (‰) for the diets of reference animals and cows milk.

Sample	**Mean (‰)**
Acorns (a)	-27.2
Acorns (b)	-24.4
Acorns (c)	-24.1
Beech nuts	-26.8
Barley fed to Baker's pigs	-24.9
Wheat fed to Baker's pigs	-24.5
Concentrate mix fed to Baker's pigs	-24.9
Concentrate mix fed to Baker's cows	-23.9
Silage eaten by Baker's cows	-28.6
Chicken feed (Edinburgh)	-25.5
Goose pellets	-26.1
Grass eaten by goose	-29.7
Grass grazed by deer at Brockley farm	-28.5
Grass grazed by cows at Brockley farm	-27.8
Grass grazed by sheep at Baker's abatoir	-31.5
Cow's milk prepared as whey (Twinkle 2)	-28.6
Cow's milk prepared as whey (Sarah)	-26.4
Cow's milk prepared as whey (Tulip)	-29.9

Table 5. $\delta^{13}C$ values[1] (‰) for fatty acids in lipid extracts from Late Saxon/early medieval vessels from West Cotton, Northamptonshire.

	Fatty acid	
Sherd no.	$C_{16:0}$	$C_{18:0}$
RP2	-26.6	-27.7
RP4	-26.9	-25.5
RP6	-27.5	-29.2
RP7	-28.9	-30.2
RP10	-26.4	-24.9
RP13	-29.0	-30.1
RP16	-28.6	-30.5
RP22	-29.4	-31.9
RP28	-26.5	-28.5
RP50	-27.9	-29.2
RP53	-28.7	-30.1
RP30	-28.5	-32.6
RP60	-29.5	-33.1
RP61	-28.4	-32.9
RP71	-28.5	-29.8

	Fatty acid	
Sherd no.	$C_{16:0}$	$C_{18:0}$
RP72	-28.3	-32.0
RP73	-26.1	-27.8
RP78	-26.5	-28.7
RP81	-29.0	-30.8
RP82	-27.7	-29.5
RP83	-26.9	-28.6
RP85	-28.3	-28.4
RP86	-28.9	-32.4
RP87	-28.1	-29.9
RP88	-26.1	-25.4
RP89	-28.1	-30.5
RP91	-27.4	-32.3
RP93	-28.2	-31.2
RP94	-29.0	-32.7
WC30	-28.3	-33.5

[1] Data has been corrected for the change in $\delta^{13}C$ of atmospheric CO_2 which has occurred since the Industrial Revolution.

Table 6. $\delta^{13}C$ values[1] (‰) for fatty acids in lipid extracts from Romano/British vessels from Stanwick, Northamptonshire.

	Fatty acid	
Sherd no.	$C_{16:0}$	$C_{18:0}$
st152	-27.8	-29.7
st153	-28.8	-31.1
st156	-29.1	-31.1
st160	-28.6	-33.2
st161	-28.6	-33.9
st190body	-27.8	-29.0
st190rim	-27.4	-28.6
st193	-28.8	-32.2
st194	-27.4	-33.2
st197	-28.6	-32.9

	Fatty acid	
Sherd no.	$C_{16:0}$	$C_{18:0}$
st206body	-28	-32.3
st206rim	-28.9	-30.2
st208	-28.4	-34.1
st210	-29.1	-31.0
st211	-29.8	-31.2
st212	-27.3	-29.7
st215	-28.2	-29.4
st226	-26.9	-28.7
st262	-27.5	-30.3

Table 7. $\delta^{13}C$ values[1] (‰) for fatty acids in lipid extracts from Middle Saxon vessels from Wicken Bonhunt, Essex.

	Fatty acid	
Sherd no.	**$C_{16:0}$**	**$C_{18:0}$**
1	-28.1	-28.0
2	-29.2	-30.0
3	-28.7	-29.3
7	-28.7	-29.7
8	-28.3	-29.0
12	-28.6	-29.8
13	-29.1	-30.5
14	-29.1	-30.5
15	-29.9	-28.0
16	-27.5	-26.9
19	-29.9	-31.1

Table 8. $\delta^{13}C$ values[1] (‰) for fatty acids in lipid extracts from early Neolithic vessels from Botai, Kazakhstan.

	Fatty acid	
Sherd no.	**$C_{16:0}$**	**$C_{18:0}$**
I POT	-27.6	-27.0
VII (N21) POT	-27.2	-27.2
(N26) POT	-27.9	-28.1
II CR	-26.2	-27.4
III CR	-27.0	-27.3

Table 9. $\delta^{13}C$ values[1] (‰) for fatty acids in lipid extracts from Siberian horse fats.

	Fatty acid	
Sherd no.	**$C_{16:0}$**	**$C_{18:0}$**
N26 pot	-27.7	-27.9
N21 pot	-27.4	-27.2
xviii pot	-26.7	-28.2

	Fatty acid	
Sherd no.	**$C_{16:0}$**	**$C_{18:0}$**
i pot	-27.6	-27.0
ii cr	-26.2	-27.4
iii cr	-27.0	-27.3

Table 10. $\delta^{13}C$ values[1] (‰) for fatty acids in lipid extracts from early-middle Iron Age vessels from Yarnton Cresswell field, Oxfordshire.

	Fatty acid	
Sherd no.	**$C_{16:0}$**	**$C_{18:0}$**
101	-28.8	-31.5
105	-28.2	-30.6
112	-28.1	-28.8
113	-27.7	-29.9
114	-29.6	-34.1
118	-28.8	-33.4
119	-30.0	-31.7
121	-28.2	-33.6
126	-28.3	-31.7
127	-28.2	-34.3

	Fatty acid	
Sherd no.	**$C_{16:0}$**	**$C_{18:0}$**
128	-27.9	-32.4
129	-27.4	-33.3
130	-27.8	-33.2
137	-29.3	-34.1
141	-29.4	-33.4
144	-28.3	-28.0
146	-28.8	-33.9
148	-28.5	-30.3
149	-30.0	-31.9

Table 11. $\delta^{13}C$ values[1] (‰) for fatty acids in lipid extracts from Neolithic/Bronze Age vessels from Yarnton flood plain, Oxfordshire.

	Fatty acid	
Sherd no.	**$C_{16:0}$**	**$C_{18:0}$**
YFPB 96		
1	-28.2	-30.7
4	-27.3	-32.6
5 (9)	-27.6	-32.4
23	-27.6	-32.3
30	-29.1	-33.8
31	-29.0	-34.1

	Fatty acid	
Sherd no.	**$C_{16:0}$**	**$C_{18:0}$**
YFP92		
38	-26.3	-25.4
YCF95		
41	-28.5	-32.7
43	-29.1	-29.9
YFP92		
49	-28.4	-31.9
50	-29.2	-34.2

Table 12. $\delta^{13}C$ values[1] (‰) for fatty acids in lipid extracts from late Neolithic vessels from Eton Lake End Road, Oxfordshire.

Sherd no.	Context no.	Small find no.	Fatty acid	
			$C_{16:0}$	$C_{18:0}$
NRA1	959	2054	-29.0	-32.0
NRA2	959	2041-8	-29.4	-33.2
NRA2	959	2041-11	-29.0	-33.5
NRA2	959	2041-12	-28.5	-33.4
NRA2	959	2041-rim	-29.0	-31.8
NRA4	1224	2271	-27.6	-28.0
NRA8	606	2163	-28.9	-32.6
NRA8	606	2164	-28.9	-30.6
NRA8	606	2166	-29.9	-32.8
NRA10	529	865	-29.5	-33.0
NRA11	685	601	-29.1	-35.1
NRA13	685	772	-29.9	-34.4

Table 13. $\delta^{13}C$ values[1] (‰) for fatty acids in lipid extracts from early Neolithic vessels from Eton Rowing Lake, Oxfordshire.

	Fatty acid	
Sherd no.	$C_{16:0}$	$C_{18:0}$
DBC1	-30.8	-33.3
DBC 3	-28.6	-33.3
DBC 7	-28.4	-33.9
DBC 8	-28.8	-33.9
DBC 9	-29.0	-34.3
DBC 11	-30.2	-34.8
DBC 12	-28.9	-31.6

	Fatty acid	
Sherd no.	$C_{16:0}$	$C_{18:0}$
DBC 13	-30.1	-32.8
DBC 20	-29.3	-32.1
DBC 21	-29.3	-33.6
DBC 22	-28.3	-33.6
DBC 25	-28.6	-32.6
DBC 28	-28.0	-33.3

Table 14. $\delta^{13}C$ values[1] (‰) for fatty acids in lipid extracts from early-late Neolithic vessels from Walton, mid Wales.

	Fatty acid	
Sherd no.	$C_{16:0}$	$C_{18:0}$
POT 5	-30.8	-32.6
POT 1a/b (mean)	-28.5	-32.4
POT 66	-27.5	-25.9
POT 3	-29.5	-33.3
POT 68	-27.8	-26.7
CR 39	-30.5	-36.3
CR 38	-29.2	-34.1
CR 33	-29.1	-33.1

Table 15. $\delta^{13}C$ values[1] (‰) for fatty acids in lipid extracts from the ethnographic vessels and modern olive oil.

	Fatty acid	
Sample	$C_{16:0}$	$C_{18:0}$
Vessel A	-23.3	-22.6
Vessel B	-27.3	-30.7
Vessel C	-26.1	-26.2

	Fatty acid	
Sample	$C_{16:0}$	$C_{18:0}$
Vessel F	-13.6	-13.7
Vessel G	-26.2	-30.3
Virgin olive oil	-28.5	-28.5

APPENDIX 7

APPENDIX 7. THEORETICAL MIXING CURVES

Table 1. Calculation of theoretical mixing curves (Woodbury *et al.*, 1995). The predicted $\delta^{13}C$ values for mixtures of cow (C) and pig (P) adipose have been shown as an example.

Proportions of fats in mixture		**Fatty acid composition (%)**				**$\delta^{13}C$ cows adipose**		**$\delta^{13}C$ pig adipose**		**Predicted value**	
Cows adipose	**Pig adipose**	**$C_{16:0}$**	**$C_{18:0}$**	**$C_{16:0}$**	**$C_{18:0}$**	**$C_{16:0}$**	**$C_{18:0}$**	**$C_{16:0}$**	**$C_{18:0}$**	**$C_{16:0}$**	**$C_{18:0}$**
C	**P**	**% in C**	**% in C**	**% in P**	**% in P**	**$\delta^{13}C_{(C)}$**	**$\delta^{13}C_{(C)}$**	**$\delta^{13}C_{(P)}$**	**$\delta^{13}C_{(P)}$**	**$\delta^{13}C_{(C+P)}$**	**$\delta^{13}C_{(C+P)}$**
100	0	27.8	20.2	24.1	11.5	-29.7	-32.2	-25.9	-24.9	-29.7	-32.2
90	10	27.8	20.2	24.1	11.5	-29.7	-32.2	-25.9	-24.9	-29.4	-31.8
80	20	27.8	20.2	24.1	11.5	-29.7	-32.2	-25.9	-24.9	-29.1	-31.3
70	30	27.8	20.2	24.1	11.5	-29.7	-32.2	-25.9	-24.9	-28.7	-30.8
60	40	27.8	20.2	24.1	11.5	-29.7	-32.2	-25.9	-24.9	-28.3	-30.2
50	50	27.8	20.2	24.1	11.5	-29.7	-32.2	-25.9	-24.9	-28.0	-29.6
40	60	27.8	20.2	24.1	11.5	-29.7	-32.2	-25.9	-24.9	-27.6	-28.9
30	70	27.8	20.2	24.1	11.5	-29.7	-32.2	-25.9	-24.9	-27.2	-28.0
20	80	27.8	20.2	24.1	11.5	-29.7	-32.2	-25.9	-24.9	-26.8	-27.1
10	90	27.8	20.2	24.1	11.5	-29.7	-32.2	-25.9	-24.9	-26.4	-26.1
0	100	27.8	20.2	24.1	11.5	-29.7	-32.2	-25.9	-24.9	-25.9	-24.9

The mathematical model of mixing is as follows:

$$\delta^{13}C_{(C+P)}=\delta^{13}C_{(C)}\{[C(\%\text{ in C})]/[C(\%\text{ in C})+P(\%\text{ in P})]\}+$$
$$\delta^{13}C_{(P)}\{[P(\%\text{ in P})]/[C(\%\text{ in C})+P(\%\text{ in P})]\}$$

where C and P represent cow and pig fats, respectively; $\delta^{13}C_{(C+P)}$ is the predicted isotope ratio of the fatty acid with contributions from both cow and pig fats; $\delta^{13}C_{(C)}$ is the isotope ratio of the fatty acid in the cow fat; $\delta^{13}C_{(P)}$ is the isotope ratio of the fatty acid in the pig fat; *C* is the concentration of cow fat present (%) ; *P* is the concentration of pig fat present (%); % in C is the amount of the fatty acid in the cow fat, and % in P is the amount of the fatty acid in the pig fat.

The theoretical mixing curves generated from this model are derived solely from the percent compositions of the individual fatty acids of the pure fats and their individual $\delta^{13}C$ values. The variations in the $\delta^{13}C$ values of the individual fatty acids with mixing are non-linear. This is due to differences in the relative fatty acid compositions of the cow (C) and pig (P) fats. Had fats been of identical fatty acid composition, linear plots of $\delta^{13}C$ value vs. percent of the different fats would have been obtained with the mixing line travelling directly from the $\delta^{13}C$ value of C to the $\delta^{13}C$ value of P.

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Direct Demonstration of Milk as an Element of Archaeological Economies

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The stable carbon isotope ($\delta^{13}C$) compositions of individual fatty acid components of remnant fats preserved in archaeological pottery vessels show that dairying was a component of archaeological economies. Characteristic $\delta^{13}C$ values arise from biases in the biosynthetic origins of the $C_{18:0}$ fatty acids in milk and adipose fat. Milk and adipose fat from animals raised on similar pastures and fodders have distinct isotopic signatures.

Although sheep are thought to have been domesticated in the Near East at ~9000 B.C. and cattle and goats were domesticated at ~7000 B.C., there is no direct evidence that they were milked. Pictorial and written records from the Sahara, Egypt, and Mesopotamia show that dairying had begun there by 4000 to 2900 B.C. (*1*). Evidence of dairying during the prehistoric period in Britain has been limited solely to secondary evidence associated with the procurement and use of dairy products, such as putative ceramic "cheese" strainers, dating from 4500 B.C. (*2*, *3*). Faunal studies have suggested that a high neonatal cull and a bias in the adult cull in domestic ruminant animals may indicate dair-

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ying (*4*, *5*). Here we provide direct evidence of dairying from preserved residues of dairy products themselves.

Degraded animal fats are the most common class of organic residue observed in archaeological ceramics (*6*–*9*), identified by characteristically high abundances of saturated fatty acids, particularly palmitic ($C_{16:0}$) and stearic ($C_{18:0}$) acids. We have searched for these residues in more than 1000 potsherds from sites throughout Europe dating from the Neolithic through Bronze and Iron Age, Roman, Saxon, and medieval periods, using gas chromatography (GC) and GC–mass spectrometry (GC/MS) techniques. Most of the vessels correspond to those generally associated with food processing or storage (that is, jars and bowls), recovered from domestic areas of settlements. More than 40% of all sherds studied yielded appreciable lipid residues (*10*–*13*). Although there is no problem in detecting degraded animal fats, identifying the origin of the fats or specifying whether they are mixtures of fats from different species is much more problematic. A number of chemical criteria can be used to distinguish between the residues of animal fats preserved in archaeological pottery (*13*), including (i) the positional isomers of monounsaturated fatty acids; (ii) the abundances of odd-carbon-number ($C_{15:0}$ and $C_{17:0}$) *iso*- and *anteiso*–branched–chain fatty acids; (iii) fatty acid and triacylglycerol distributions; and (iv) the $\delta^{13}C$ values of the major saturated fatty acids $C_{16:0}$ and $C_{18:0}$, determined by GC–combustion-isotope ratio MS [GC-C-IRMS (*13*, *14*)]. Bulk stable isotope studies allow the detection of remnant fats in carbonized food residues from archaeological sites (*15*, *16*), but compound-specific $\delta^{13}C$ measurements can allow the fats from ruminant and nonruminant animals to be distinguished (*13*).

One major category of fat that we should be able to detect in pottery vessels is that derived from milk (for example, butterfat). Just as with the adipose fats, the processing of milk by pasteurizing or cooking, involving milk or butter, would result in the absorption of appreciable quantities of fat into the walls of unglazed pottery vessels. Fresh milk fats differ from adipose fats in their fatty acid composition because of the presence of short-chain (C_4 to C_{14}) saturated fatty acids (*17*). However, even though it is known that dairying was widely practiced in the Roman and later periods, we have consistently failed to detect fatty residues containing these characteristic shorter-chain fatty acids in lipid extracts from pottery vessels.

Because dairy products must have been processed in pottery vessels (*18*, *19*), our apparent inability to detect dairy fats must stem from compositional alteration through decay during burial. As a test, we examined the triacylglycerol distributions of milk fat from fresh milk and of ruminant adipose fat after decay in the laboratory when absorbed into unglazed replica ceramic sherds (Fig. 1). Over a short time, the distribution of lipid components in milk transformed into a distribution more closely resembling that of the adipose fat through preferential hydrolysis of the short-chain acyl moieties as a result of reduced steric effects at ester linkages in triacylglycerols as compared with their long-chain counterparts (*20*). Once released from triacylglycerols by hydrolysis, the short-chain fatty acids are appreciably more water soluble (and volatile) than their long-chain counterparts (*21*). This experiment confirms that selective decay of milk lipids leads to a distribution of fatty acids resembling that of adipose fats. Hence, although the fats preserved in archaeological pottery in principle offer an excellent source of information concerning the exploitation of dairy products by early farmers, our ability to recognize them through chemical analysis has been thwarted because of diagenetic alteration during burial.

The $\delta^{13}C$ values are diagnostic, however. We analyzed by GC-C-IRMS fats extracted from

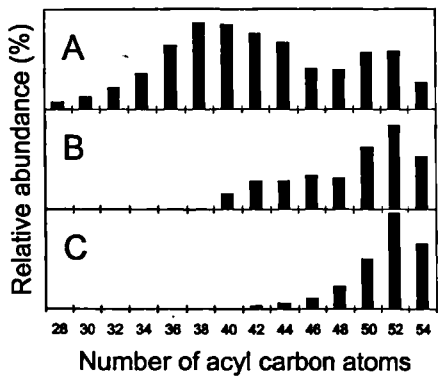

Fig. 1. Triacylglycerol distributions in (A) fresh milk, (B) milk absorbed in an unglazed potsherd and degraded in the laboratory under oxic conditions for 90 days, and (C) fresh ruminant (ovine) adipose fat. The distributions were determined by high-temperature GC of total lipid extracts (*23*). Clean replica potsherds (approximately 2 g) were soaked in solutions of White goat milk, with absorption facilitated by ultrasonication (2 by 20 min). Potsherds were dried to constant weight at room temperature before burial in flasks of mushroom compost. The flasks were plugged with extracted cotton wool to allow diffusion of air and then incubated at 30°C.

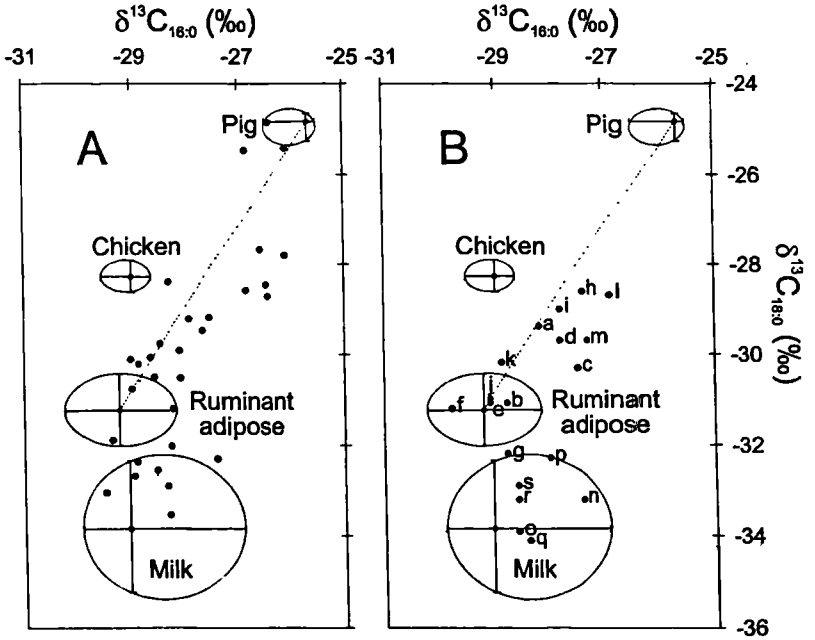

Fig. 2. Plot of the $\delta^{13}C$ values of the major *n*-alkanoic acid components ($C_{16:0}$ and $C_{18:0}$) of the lipid extracts from potsherds from (A) West Cotton (late Saxon to early medieval) and (B) Stanwick (Iron Age–Romano-British). The archaeological fats (solid circles) cluster near the reference adipose and milk fats (bovine and ovine). In the case of West Cotton, nonruminant (porcine) adipose fats have also been identified. The mixing curves (dashed lines) have been calculated (*25*) to illustrate the $\delta^{13}C$ values that would result from the mixing of ovine/bovine and porcine fats in the vessels. The encircled fields encompass the ranges for reference animal fats, with the ranges crossing at the arithmetic mean. The numbers of different reference fats analyzed were as follows: pig adipose fat, 4; ruminant adipose fat, 9 (3 cow and 6 sheep); chicken adipose fat, 8; milk fat, 7 (6 cow and 1 sheep). All the animals were raised on C_3 diets. The more depleted $\delta^{13}C$ values for the C_{18} fatty acid in the milk fats arises through routing of a large proportion of fatty acids directly from the diet (after biohydrogenation) to milk production. The $\delta^{13}C$ values for the fatty acids in the reference fats have been corrected for the post–Industrial Revolution effects of fossil fuel burning, which has decreased the $\delta^{13}C$ value of atmospheric CO_2 by 1.2‰ since the middle of the 19th century (*26*). The letters adjacent to the points in (B) correlate with the triacylglycerol distributions shown in Fig. 3 and correspond to the following types of domestic archaeological vessels: a, b, e, f, h through m, p, r, and s are jar-form vessels of various sizes; c is a mortaria; d is a ceramic lid; g and n are flanged and wide bowls, respectively; and i, o, and q are small dishes. There was no obvious correlation between vessel form and the type of fat they contained. Analytical precision is ±0.3‰.

pottery vessels recovered from the late Saxon to early medieval site of West Cotton, Northamptonshire, UK, and from the Iron Age–Romano-British site of Stanwick, also in Northamptonshire, and compared the results with those from ruminant and nonruminant modern reference fats (Fig. 2). At West Cotton (Fig. 2A), the archaeological fats cluster in the range of the ruminant and nonruminant reference fats, whereas the extracts from Stanwick (Fig. 2B) are biased toward ruminant fats, and several analyses indicate mixtures of ruminant and nonruminant adipose fats and appear to show that different fats were processed in the same vessels.

In several of the ancient fats, the $C_{18:0}$ fatty acid is depleted in ^{13}C as compared with the other remnant fats obtained from the archaeological vessels. Consideration of the biochemistry and physiology of milk production in ruminant animals (*17*, *22*) and subsequent analysis of reference milk fats obtained from sheep and cattle reared on a C_3 pasture indicate that these ^{13}C-depleted fatty acids originate from milk fat. The $\delta^{13}C$ values for the reference milk fats plot close to the values obtained for the archaeological fats containing the more depleted C_{18} fatty acids.

The distinctive trends seen in the $\delta^{13}C$ values of the dairy product $C_{16:0}$ and $C_{18:0}$ fatty acids reflect their different biosynthetic origins. The $C_{16:0}$ component in milk is synthesized largely in the mammary gland de novo from acetate (derived mainly from dietary carbohydrate). The $C_{18:0}$ component derives in part directly from the dietary fatty acids, mainly $C_{18:2}$ and $C_{18:3}$, by biohydrogenation (bacterial reduction) in the rumen and in part from other sources, such as mobilization of adipose fatty acids (Fig. 3). During lactation, it has been found that ~40‰ of milk fat is derived directly from absorbed dietary fatty acids, corresponding mainly to C_{18} components (*17*, *22*). In ruminant adipose fats, the $C_{16:0}$ and $C_{18:0}$ fatty acids are also derived from a combination of dietary lipids and de novo synthesis; however, the enhanced routing of dietary fatty acids directly to milk during lactation gives rise to the more negative $\delta^{13}C$ values for the $C_{18:0}$ fatty acid of milk fat as compared with the adipose fat of animals feeding on the same diets. The more negative $\delta^{13}C$ values [~ 32.5 to 35 per mil (‰)] seen for $C_{18:0}$ in milk is similar to the depleted values recorded for the major unsaturated C_{18} fatty acids in pastures and fodders: –35‰ (the weighted mean for C_{18} fatty acids in grass). Thus, our data show that milk and adipose fats from animals raised on similar diets are separable on the basis of the comparison of the $\delta^{13}C$ values of $C_{16:0}$ and $C_{18:0}$ fatty acids and that this provides the basis for determining the presence of milk fat in archaeological pottery. The trend is seen for both cows and sheep because the mean value obtained for the reference cows' milk $C_{18:0}$ fatty acid was 34‰ and that of the sheep's milk was 33.8‰.

The triacylglycerol distributions (Fig. 3), derived by high-temperature GC (*23*), for the extracts in Fig. 2B provide further information. Those displaying the heavier $\delta^{13}C$ values for their C_{18} fatty acids more closely resemble the distribution characteristic of fresh adipose fat (Fig. 1C) than that of either fresh (Fig. 1A) or degraded (Fig. 1B) milk fat. The distributions for the vessels yielding the lighter $\delta^{13}C$ values show a remarkably close correspondence to the laboratory-degraded milk fat (Fig. 1B). Although the triacylglycerol distributions support the isotopic differences between the various animal fats, the $\delta^{13}C$ values offer a more robust criterion because intact triacylglycerols are frequently not preserved in ancient pottery as they are completely hydrolyzed to their component free fatty acids.

Because the trends seen in the $\delta^{13}C$ values of the individual fatty acid components of animal fats reflect the fundamental difference in the $\delta^{13}C$ values of the fatty acids and carbohydrate components (~5‰) of typical forage and fodder materials (*24*), this method can be applied at any archaeological site.

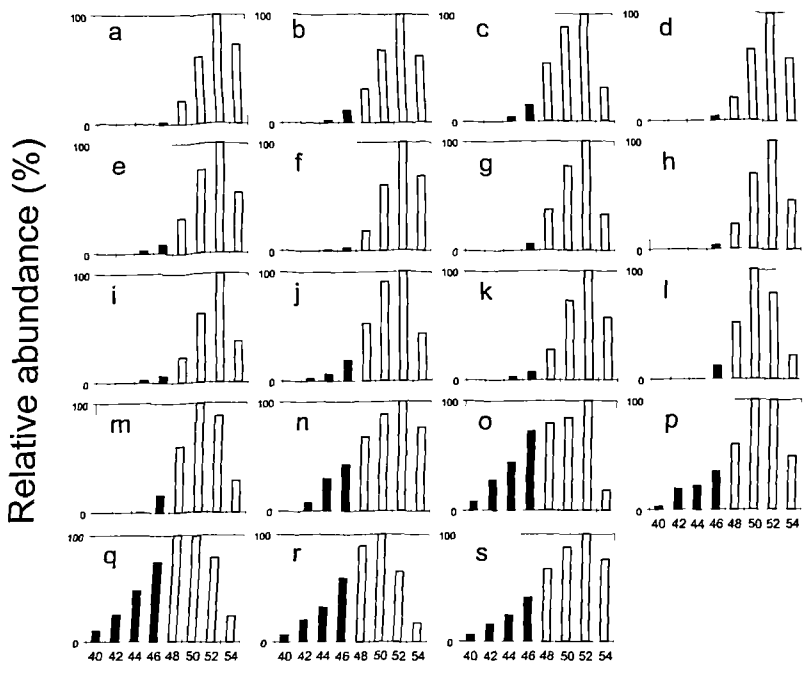

Fig. 3. Histograms showing the distributions of triacylglycerols of equal carbon number in total lipid extracts from potsherds from the Iron Age–Romano-British vessels recovered from excavations at Stanwick. Distributions a through m correspond to those displaying $\delta^{13}C$ values characteristic of ruminant adipose fats, whereas n through s correspond to those displaying $\delta^{13}C$ values characteristic of milk fat. The identification of the origins of these fats is supported by comparison with the triacylglycerol distributions, notably the higher abundance of C_{40} to C_{46} components (solid bars), which characterize degraded milk fat (compare with Fig. 1B).

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